Comptes rendus du 40e atelier annuel sur la toxicologie aquatique : du 6 au 9 octobre 2013, Moncton, Nouveau-Brunswick

Proceedings of the 40th Annual Aquatic Toxicity Workshop: October 6-9, 2013, Moncton, New Brunswick

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Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

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Fisheries and Aquatic Sciences 3098

2014

PROCEEDINGS OF THE 40TH ANNUAL AQUATIC TOXICITY WORKSHOP: OCTOBER 6-9, 2013, MONCTON, NEW BRUNSWICK

Editors:

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Preface

The 40th annual Aquatic Toxicity Workshop (ATW) was held at the Delta Beauséjour in Moncton, New Brunswick from October 6 to 9, 2013. The Workshop included two plenary presentations, 65 platform and 33 poster presentations, and special panel discussions on 'Ecotoxicology 101' and on unconventional oil and gas activities. Total attendance was 183.

This workshop was one of a continuing series of annual workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These workshops are administered by a Board of Directors and organized by local organizing committees. The Proceedings are published with the support of the Department of Fisheries and Oceans.

Préface

Le 40ième Atelier annuel sur la toxicité aquatique a eu lieu au Delta Beauséjour à Moncton, Nouveau-Brunswick, du 6 octobre au 9 octobre 2013. L'atelier a donné lieu à deux communications lors de séances plénières, 65 présentations orales et 33 présentations par affiche, et des tables rondes sur les principes fondamentaux de l'écotoxicologie et sur les activités liées à l'industrie du pétrole et du gaz non conventionnels. Cent quatre-vingt-trois personnes ont assisté à l'atelier.

L'atelier a permis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces ateliers annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations publiques et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés avec le soutien du ministre des Pêches et Océans.

Editors' comments

This volume contains papers, abstracts, or extended abstracts of all presentations at the workshop. An author index is also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. In most cases, the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The editors would like to thank Dr. Jill Watson for her assistance in preparing these proceedings.

Remarques des éditeurs

Ce compte rendu renferme le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs est aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La longueur et la forme des communications varient parce que ces dernières sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elles ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

Les rédacteurs voudraient remercier Jill Watson dans la préparation de ces comptes rendus.

40th Aquatic Toxicity Workshop Organizing Committee / Comité organisateur du 40e atelier de toxicologie aquatique

Workshop Co-Chairs / Co-présidents de l'atelier:

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Les Burridge	Burridge Consulting Inc.

Workshop Organizing Committee / Comité organisateur de l'atelier:

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PO – Poster presentation

Forty Years of ATW, 1974-2013

The following article is a summary of a presentation given by Gordon Craig at the 40th annual Aquatic Toxicity Workshop (ATW) in Moncton, New Brunswick. Gordon chaired the first ATW, in its current form, in 1975 after precursor meetings in Toronto and Winnipeg over the two previous years. He has since chaired or co-chaired three ATWs and has been continually involved in the evolution of the ATW. Once a governing Board of Directors was created in 1984, Gordon rotated on and off the Board until he was appointed a long-term Director in 2003. Because of his intensive involvement since the ATW's inception, Gordon was well positioned to prepare this retrospective: he has attended all but a couple of the ATWs over his long and productive career in ecotoxicology.

G.R. Craig[1]
[1] G.R. Craig & Associates

Introduction

The Aquatic Toxicity Workshop (ATW) has been active for 40 years since the first formal meeting of aquatic toxicologists from academia, government, industry, and the consulting sector took place in Winnipeg in 1974. Aquatic toxicology had begun decades before in small pockets of academia and government in the U.K, United States, and Canada. The primary locations in Canada were in the Maritimes, Quebec, Ontario, and British Columbia. It was early leadership within Environment Canada (EC) and the future Department of Fisheries and Oceans (DFO) that led to what would become ATW.

This reflection on the beginnings and development of ATW focuses on events and individuals who shaped the evolution of the workshop and identifies how it has changed over the years. The workshop has taken on a national purpose to provide a platform to discuss and debate environmental regulatory changes, introduce new testing and data analysis technologies, and report on the quality of the Canadian environment in the moment and over time.

The fundamental flexibility of the organization has enabled ATW to endure. Once ATW was incorporated in 1984, a formality of organization developed that allowed environmental researchers and managers from across Canada, among all sectors, to share their experiences and views on how to protect and preserve our natural environment.

Much of the material following has been documented in the Proceedings of the Annual Aquatic Toxicity Workshop as part of the *Canadian Technical Report of Fisheries and Aquatic Sciences* series. All the proceedings from past years have been converted to a PDF format and are available on the ATW.CA website (Aquatic Toxicity Workshop 2014).

Before ATW

A number of individuals were involved in conducting the fish toxicity tests and pollution assessments of the early 1950s and 1960s that John Sprague reviewed at the St. Andrews workshop

(1996). Peter Wells is publishing a thorough review (in press) of environmental science at the St. Andrews Biological Station from 1908 to the 1990s, identifying studies and project scientists operating out of that station during that time. The following are a few of the scientists who were conducting toxicity tests and studies across Canada before the establishment of ATW.

Don Alderdice conducted assessments on the effects of DDT spraying on salmon fry in the Maritimes through the early 1950s and then moved to the Nanaimo Biological Station in British Columbia. He reported on the response of salmon fry exposed to insecticides and pulp and paper effluents throughout the decade.

John Neal, working for the Ontario Department of Health in the early 1950s, focused on the issues involving the Kalamazoo Vegetable Parchment Company (KVP) in Espanola, where an old Abitibi Power and Paper mill was being reactivated after the war. The Spanish River had recovered from the Abitibi mill shutdown in 1930 and had become a thriving sport fishery with tourist camps along the river to Georgian Bay. When KVP restarted mill operations in 1946, the Espanola River quickly became unfit for drinking and fish life. John Neal set up a fish toxicity lab within the Ontario Water Resources Commission (OWRC) laboratory at Rexdale, Ontario. It went through major technological upgrades in the early 1970s as the Commission transformed into the Ontario Ministry of Environment (OMOE).

John Sprague was one of the early workers to introduce the principles of pharmacology to the practice of aquatic toxicity testing in the mid-1950s while working on his PhD with the famous Fred Frye at the University of Toronto. After graduation, he worked at the St. Andrews Biological Station, where he studied pulp mill and mining effluent impacts on Maritime rivers. Some studies from the St. Andrews Biological Station on DDT and its toxicity to salmon were cited in Rachel Carson's (1962) *Silent Spring*.

Gérard Leduc conducted work with the Quebec Biological Bureau and later taught at Sir George Williams University (now Concordia University), supervising one of the early toxicity laboratories and focusing on the effects of cyanide and metalocynides on fish. Gérard was a Co-Chair of the 7th ATW in Montreal in 1980. Perry Anderson, also from Sir George Williams University, conducted work in the mid- to late-1970s on metal speciation and the toxicity of metal mixtures to fish.

Terry Howard arrived from Britain and joined Don Alderdice at Nanaimo in the early 1960s, then moved on to B.C. Research to study the lethal and sublethal effects of pulp mill effluents on fish through the 1960s. B.C. Research was the pulp and paper industry's centre for process design, effluent treatment, and studies of environmental impacts from the 1960s to the 1980s.

James Servizi worked in the toxicity laboratory in Cultus Lake, B.C., for the International Pacific Salmon Commission from 1963 to 1993. It was his 1966 work (Servizi et al. 1966) that was used to derive the first pulp and paper effluent regulatory toxicity test, promulgated in 1971, of 80% rainbow trout survival in a 65% dilution of effluent over 96 hours of continuous flow exposure.

By the mid-1970s (Table 1), there were 18 fish toxicity-testing laboratories across Canada. The majority were federally operated by EC or DFO; several industry research institutes, a couple of universities, and consultancies also operated labs.

Table 1. Aquatic toxicity laboratories of the 1970s

Federal	• Environmental Protection Service (EPS) – Halifax, Winnipeg, Burlington, Edmonton, Vancouver, Yellowknife • DFO – Halifax, Winnipeg, Burlington, Nanaimo, St. Andrews
Provincial	• OMOE • B.C. Pollution Control Branch
Research Institutes	• Noranda • International Pacific Salmon Fisheries Commission • B.C. Research
Universities	• Sir George Williams University – Montreal • University of Toronto • University of Guelph
Consultants	• BEAK Consultants – Toronto • Enviroclean – Toronto

Biological toxicity became a measurable and enforceable parameter from 1971 onwards as a result of the new *Pulp and Paper Effluent Regulations* and guidelines under the revised Canadian *Fisheries Act*. The continuous flow testing protocol in the regulations was logistically limiting. Even though every pulp mill had to comply, few mill effluents were tested and reporting was not required.

The technical requirements of continuous flow testing were prohibitive for all but federal laboratories with on-site testing facilities. Among the challenges were that large volumes of effluent (several 45-gallon drums or tank truck loads) had to be collected and delivered to the testing lab; there were still major debates as to whether to aerate the sample during testing or not; fish size requirements were less than 2 g; no specific standard method was employed: although a number were available from ASTM and Standard Methods (American Public Health Association 1961), those were largely for static tests and not for rainbow trout. As a result, there were issues about comparability of results between laboratories and over time. Reproducibility, the cornerstone of science, could not be assured in toxicity testing by regulators.

Communication among agencies in the 1970s was difficult by today's standards and was limited to phone calls, letter writing, faxing, and few, if any, conferences. No conferences focused on aquatic toxicity because it was a relatively new discipline, but some toxicity studies were reported at American Fisheries Society and North American Benthological Society meetings. Those societies covered a wide range of topics and held their annual meetings in the United States, which was limiting for most Canadian regulatory agency staff because of the international travel designation.

Water Quality Criteria – Driving the Need for Standard Methods

The need for standardized testing methods evolved with a drive to develop surface water quality criteria, guidelines, and objectives. The United States passed the *Water Quality Act* in 1965, requiring individual states to develop water quality criteria, and the U.S. Environmental Protection Agency (EPA) began to take a lead with various compendium documents. The first U.S. water quality

guidelines document appeared in 1972 (National Academy of Sciences 1972), the second in 1976 (Environmental Protection Agency 1976). Ontario published its initial water quality criteria in 1967 and updated and expanded its policy between 1970 and 1994 (Ontario Water Resources Commission 1967, 1970; Ontario Ministry of the Environment 1978; Ontario Ministry of the Environment and Energy 1994). EC published its *Guidelines for Surface Water Quality* in 1979 (Environment Canada 1979a, 1979b) and continued water quality guideline development work through the Canadian Council of Ministers of the Environment (CCME), which was created in 1983 and continues to exist today. All of these documents relied on reports of metal or chemical toxicity to fish and invertebrates, and the methods used varied by researcher.

Not surprisingly, aquatic toxicity test method standardization and theory developed concurrently with water quality criteria development. John Sprague published a series of three articles from 1969 to 1971 titled "Measurement of pollutant toxicity to fish" (Sprague 1969, 1970, 1971), the first of which was named a citation classic in 1979. He followed those in 1973 with the "ABCs" of pollutant bioassays (Sprague 1973). Charles Stephan published an EPA acute toxicity test methods manual (Environmental Protection Agency 1975), the product of a committee that had been meeting since 1971. Generic toxicity test methodology was well established but needed refinement to be reproducible, allow better understanding of the mechanisms of toxicity, and be adaptable to local and national requirements.

The emerging need through the 1960s and 1970s was for measurement of the aquatic toxicity of specific "toxic chemicals" in water and industrial effluent discharges to surface waters. The management of the quality of freshwater and marine waters with these tools was the backdrop to the formation of the annual ATW.

Early Workshops

The first meeting of aquatic (environmental) toxicologists and environmental managers in Canada was hosted in 1973 by BEAK Consultants in Toronto (BEAK Consultants Ltd. 1973). The pulp and paper regulatory toxicity test, a cumbersome continuous flow method, was in place and there was concern from industry as to where all of this was going. The meeting held in Rexdale, a suburb of Toronto, attracted 195 attendees from government, academia and industry. Among the six key presenters were John Cairns from Virginia Polytechnical Institute, John Sprague from the University of Guelph, and John Loch from the Fisheries and Marine Service (part of the Department of the Environment that later became DFO) in Winnipeg. Topics touched on methods, selection of test species, how best practicable treatment fit with regulations, and the need for effective communication between biologists and engineers.

The following year (1974), John Loch, together with John Davis of the Fisheries and Marine Service in Vancouver, initiated what became the first ATW meeting, to which people involved in aquatic toxicity testing across Canada were invited. Held in Winnipeg, its purpose was to describe the different facilities and capabilities in labs across Canada and to compare the available technical approaches to bioassay testing of industrial wastes and micro-contaminants.

The Winnipeg meeting, which attracted about 40 people, was a first formal networking opportunity among academics, regulators, and the private sector in the field of aquatic toxicology in Canada. The meeting represented the recognition that another means of regulating industry would be fish toxicity testing in addition to chemical limits. No one at the time had any idea of the enormity of the undertaking or the diverse directions in which it would lead. The focus was on how to measure acute lethality of substances to fish.

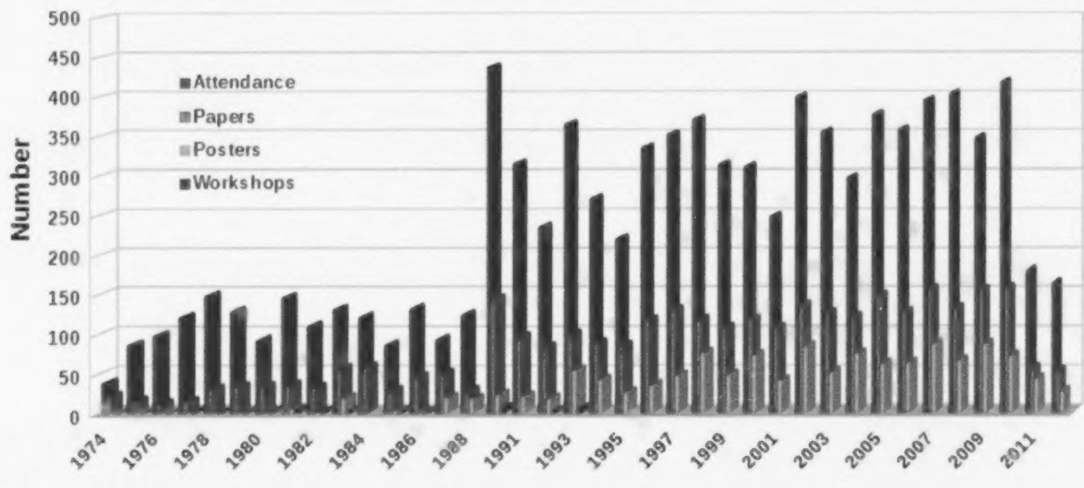
The second ATW meeting was hosted in 1975 by the Ontario Ministry of the Environment (OMOE) in Rexdale at the Resources Road laboratory, with almost 90 attendees, and was chaired by Gordon Craig. As papers and discussions unfolded, the term "workshop" recurred. When it came to publication of the proceedings, the term was included in the title, "Aquatic Toxicity Workshop." The name has endured, but, as the governance of the incorporation has evolved, there has been increasing discussion toward having the name be more inclusive of the ecological framework in which aquatic toxicology is conducted.

The third ATW was hosted in Halifax in 1976 by Environment Canada (EC) and attracted over 100 people, including some distinguished contributors to the field such as Peter Douderoff, John Sprague, and John Davis, who led a panel discussion that revolved around the standardization of regulatory tests and test methods in general. This meeting was the one that exhibited momentum. A contingent from Vancouver volunteered to host the 1977 ATW, and the annual meeting was on a roll.

ATW's Growth and Development

Subsequent meetings of ATW over the first 14 years quickly attracted between 125 and 150 attendees annually as the venues cycled across the country to eastern, central, and western locations. The 1990 Vancouver meeting experienced a huge jump in attendance to 440 as a result of Peter Chapman's strong promotion of the workshop on the west coast and into the U.S. That year's attendance set a record that has been closely approached but not yet exceeded (Figure 1). Attendance levels between 300 and 400 continued over the years until 2011, when the effects of the 2008 global economic collapse were fully felt. The impact on ATW was that attendance dropped to pre-1990 levels in 2011.

Figure 1. Numbers of attendees, papers, posters and workshops over 40 years



Presentations throughout ATW have always been abundant, with 47% of attendees making either platform or poster presentations on average over all years. The presentation of posters did not really take hold until 1987, although there were several earlier years with some posters (most with none). Once established, poster presentations comprised about a third of total presentations. Mini-workshops and panel discussions were more popular prior to 1994, at which time they dropped off the program (Figure 1). The 2012 membership survey indicated an interest in re-instating workshops.

Half- and full-day short courses on many different topics were introduced in 1996, either in classroom, local laboratories, or field settings. They continue based on the interest of instructors stepping forward and the program plans of the local organizing committee.

The workshop has reflected the pressing issues and needs of the day through its program and by providing a venue for complementary groups—the CCME working group developing multi-media quality objectives, the Inter-Governmental Ecotoxicological Testing Group (IGETG) developing national test methods, Environmental Effects Monitoring (EEM) technical working groups, oil sands working groups and international working groups, which often held meetings in conjunction with ATW.

Local Organizing Committees

Every year, the ATW Board of Directors canvasses for potential Chairs of future meetings, usually approaching individuals two to three years in advance. Chairs or Co-Chairs then engage colleagues to form a local organizing committee to plan and manage the workshop. In 2002, Peter Chapman authored the first version of the Board's standard operating procedure (SOP) to assist local organizing committees in setting schedules to complete tasks so they would benefit from lessons learned in the planning and implementation of past workshops. The SOP has been updated annually based on recommendations from each retiring organizing committee. The organizing committee has complete autonomy to invite guest speakers, promote key issues and set the theme of the workshop.

Over the past years, 49 individuals have chaired an ATW, with a few serving as chair a number of times (Table 2). Over 450 people have participated on organizing committees (Appendix 1) during that period. The commitment of chairs and organizing committee members has enabled the workshop to continue year after year.

ATW has established a loyal following, enabling the network of individuals working within public and private sectors to address issues of standardized toxicity tests, water quality guidelines, regulatory requirements of resource industries and the exploration of new mechanisms of aquatic toxicology.

Table 2. Individuals chairing multiple ATWs

Three-term Chairs	**Institution**
Raymond Van Coillie	EC
Gordon Craig	OMOE / BEAK Consultants
Two-term Chairs	
Earl Baddaloo	Alberta Environment
Les Burridge	DFO
John Davis	Fisheries and Marine Service (DFO)
Karen Mathers (Honorary)	Tetr*ES* / Stantec
Scott Munro	Sarnia-Lambton Industrial Environmental Association
Graham van Aggelen	EC
Peter Wells	EC / Dalhousie University

Governance

Until 1980, individual institutions initiated the offer to host and took all the financial risks of conducting the conference. The preceding Chair of the workshop passed on materials and suggestions for the logistics of the next meeting, but that was all.

In 1980, Sharon Leonard (DFO), who served on the Winnipeg 1979 organizing committee, proposed that a continuity fund be established to underwrite advance hotel booking costs and various other obligations that were becoming necessary to launch subsequent workshops. Many of the past and future hosts were either federal or provincial government institutions whose organizing committees were very conservative in managing finances. The result was that surplus funds were accumulating and could only be passed on to subsequent workshops through sponsorships.

The formation of a separate organization solved the complexity of advances and financial transfers, and enabled academic and private sector groups to participate in hosting. Sharon Leonard drafted and filed federal not-for-profit incorporation articles under the name "The Canadian National Aquatic Toxicity Workshop," which was registered in 1984. Incorporation also required a Board of Directors, and, as no one was looking for a lifetime appointment, a rotation mechanism was worked out. The Board would have a Continuity Chair, held initially by EC (Keith Marshall and, later, Mike Gilbertson, both from the Canadian Wildlife Service), then by a DFO representative when Mike Gilbertson moved to DFO. Peter Wells (EC), on assignment in Ottawa at the time, worked with Mike to arrange for DFO to

provide continued support for the ATW series. The other directors would be the Chairs of the previous, current, and following year's workshops. Funds, held in a separate ATW corporate bank account, would be managed by the Continuity Chair, who also edited and published the proceedings as part of a DFO technical report series. Directors would rotate on and off the Board on a three-year cycle.

ATW was productively governed in this way for the next 22 years (Figure 2) and began to take on additional responsibilities. The continuity fund was growing, investment decisions were made, the student program awards were financed and student Board members' costs were underwritten by the fund to foster succession in the long term.

In 2002, the Board decided that more permanence was required to assist the Continuity Chair and strengthen the corporate memory of ATW. The designation of "Long-term Director" was created with an indeterminate term, and that position would be appointed by the board. The other directors would rotate through as before. Art Niimi was the Continuity Chair at the time, and Scott Munro and Gordon Craig became the first long-term directors.

Seven individuals have held the position of ATW Continuity Chair since conception, with Art Niimi having the longest standing of 19 years (Table 3). DFO supported ATW by allocating a portion of the Continuity Chair's professional time to managing the organization and editing and publishing the proceedings. Before word processors and desktop computers became part of the office landscape in the 1980s, publishing the proceedings was a substantial task.

Figure 2. Evolution of ATW governance over 40 years

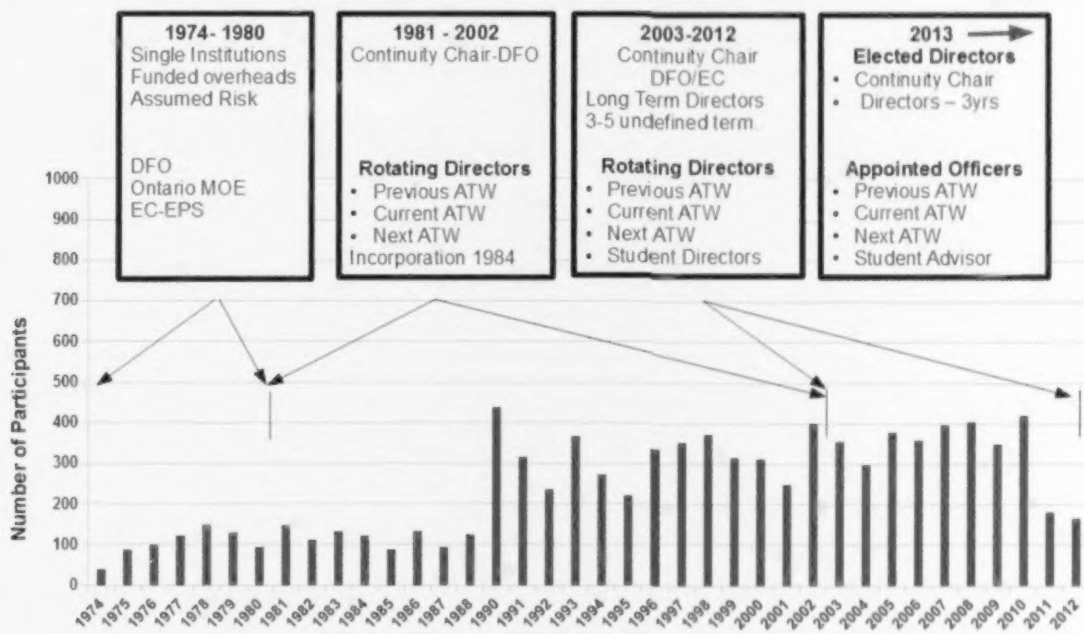


Table 3. ATW Continuity Chairs

Continuity Chair	Years of Office	Duration
Sharon Leonard (Honourary)	1979 – 1980	2 years
Keith Marshall	1980 – 1981	2 years
Mike Gilbertson	1981 – 1986	5 years
N.Y. Khan	1986	1 year
Art Niimi	1986 – 2005	19 years
Les Burridge	2005 – 2011	6 years
Rosalie Allen Jarvis	2011 – present	3 years

New federal legislation came into force in 2011 that required a number of changes to the governance of "not-for-profit" corporations, which had a direct effect on ATW. Key changes included election of directors by the membership, the appointment of a public accountant, annual reporting to Corporations Canada and Canada Revenue Agency, and making year-end financial statements available to the ATW membership. Rosalie Allen Jarvis took on the Herculean task of creating the first set of ATW articles and by-laws in 2012 to meet the new legislative requirements.

Members at the 2013 meeting in Moncton, at which the first election of directors was held, approved new articles of incorporation and by-laws. Directors were elected for a three-year term and officers of the corporation, representing chairs of past, current, and future ATW organizing committees, as well as graduate student advisors, would be appointed by the Board. The officers would rotate on a three-year cycle as they had done as directors previously. The first directors elected to the corporation in 2013 were Rosalie Allen Jarvis, continuing in her role as Continuity Chair, Gordon Craig, and Dave Huebert. Les Burridge was subsequently appointed by the Board as a fourth director for a one-year term.

The Aquatic Toxicity Workshop has been ahead of many other environmental organizations over the years. It formed three years before the Annual Symposium on Aquatic Toxicity of the American Society for Testing and Materials (ASTM), six years before the Society of Environmental Toxicity and Chemistry (SETAC), and did so without membership fees, significant administrative overhead, or a subsidy from established institutions. It was among the first conferences to create a web site and an online administration program, which was first established to help manage the 1997 workshop at Niagara Falls. ATW has managed largely on the volunteer efforts of environmental scientists, managers, academics and students across Canada. The creation of the continuity fund to support organizing committees and the student program has made the organization fully self-sustainable.

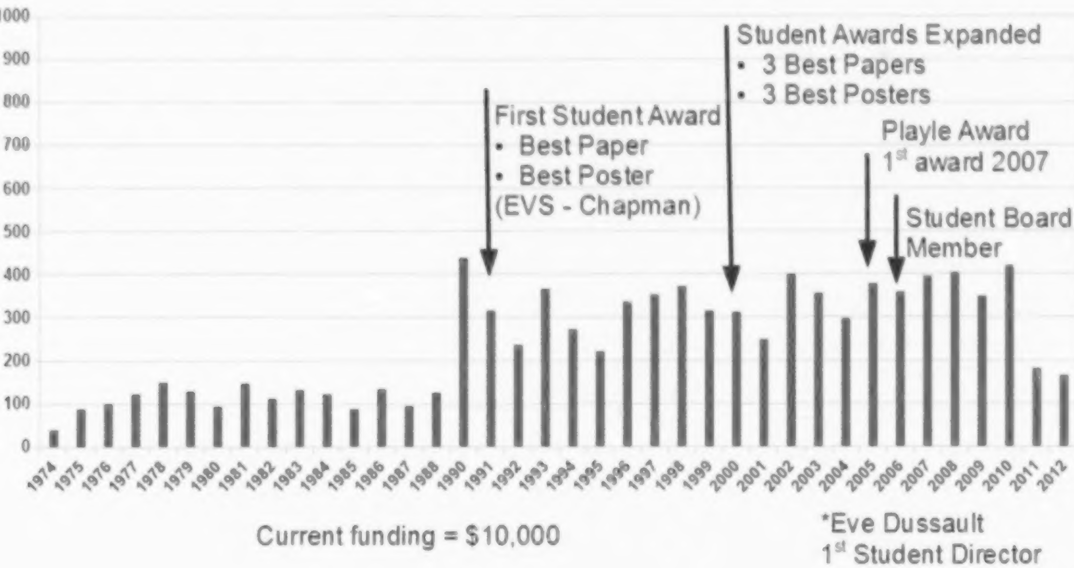
Student Program

The ATW Board established a student program, beginning in 1991 with an initiative by Peter Chapman offering an EVS Consultants award for best student paper and poster (Figure 3). The Board

assumed the continuation and development of the student awards program thereafter, increasing the awards to three for each of platform and poster presentations with cash prizes in 2000. In 2005, the Waterloo organizing committee created the Playle Award in memory of Dr. Richard Playle, of biotic ligand fame, that recognized outstanding theses for both Honours B.Sc. and M.Sc. graduates. Winners of the award present their work at the subsequent ATW; their travel expenses and one day's registration are covered by the Board.

In 2006, the Board created two student positions to foster interest in the governance of ATW. Student advisors receive full registration and travel costs for their term. The positions are staggered two-year terms so one student rotates on and another rotates off as officers of the corporation each year. Currently, the level of financial support for the student program through the presentation prizes, the Playle award, and student board representation totals about $10,000.

Figure 3. Chronology of the ATW student program



Important Events

EC Toxicity Technical Committee and the Inter-Governmental Aquatic Toxicity Group

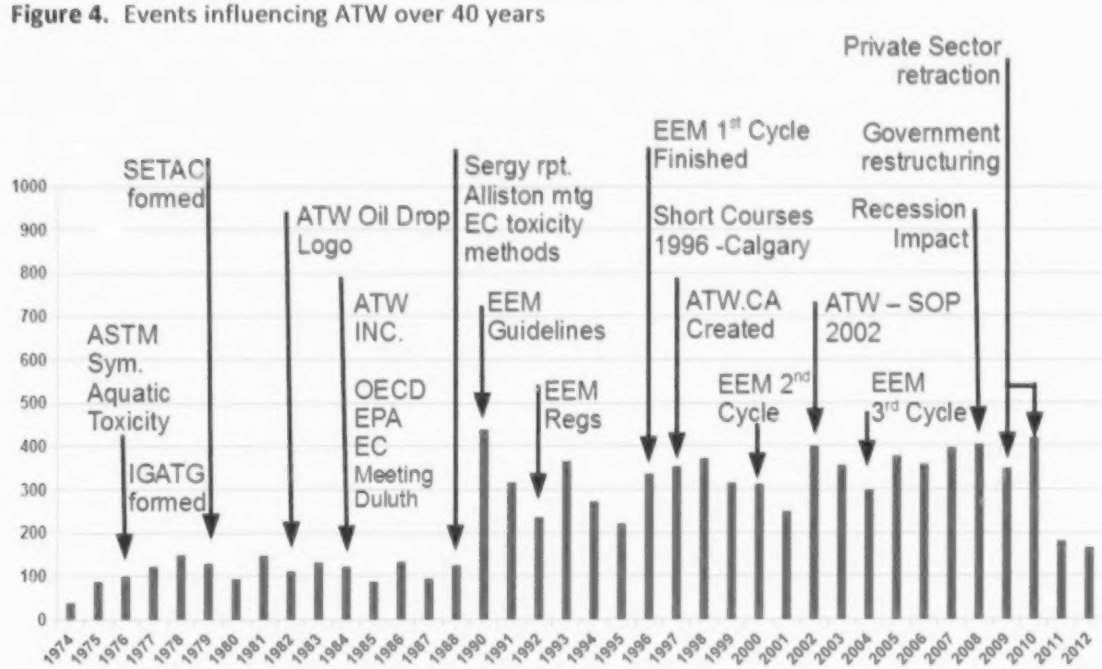
ATW was shaped by many important events during its history (Figure 4). Two such parallel events were the formal creation of a Toxicity Technical Committee within EC–EPS in 1976 and an informal meeting of provincial and federal laboratory managers, which began about the same time or shortly after.

The Toxicity Technical Committee was internal to EC and initially chaired by Ed Pessah, out of the Dartmouth facility. It was formed to advise on technical matters related to effluent regulations and guidelines under the *Fisheries Act*. Trout toxicity test methods had already been published in effluent

regulations and guidelines under the *Fisheries Act* for the pulp and paper (Environment Canada 1971), petroleum (Environment Canada 1974), metal mining (Environment Canada 1975), textile (Environment Canada 1976) and alkali products (Environment Canada 1978) sectors. The test procedures at the time were not only technically problematic, being continuous flow, but could only be updated by revising the regulations or guidelines, which was administratively cumbersome. There was a need for a more flexible solution.

The managers of federal and provincial government aquatic toxicity laboratories in Dartmouth, Quebec City, Montreal, Toronto, Burlington/Hamilton, Winnipeg, Edmonton, and Vancouver began to meet independently because they were actually using the tests and were experiencing the difficulties first-hand. This group addressed loading rates in acutely lethal rainbow trout tests (Craig and Beggs 1978) and, later, reference toxicant applications (Craig and Holtze 1981). In 1978, Art Beckett (EC, Edmonton) and Gordon Craig (then with the OMOE) decided to profile their meetings over the last several years and identify the work underway. They prepared a brief poster for the 1978 ATW in Hamilton and created the title Inter-Governmental Aquatic Toxicity Group (IGATG).

Figure 4. Events influencing ATW over 40 years



The interests within the informal IGATG and the formal EPS Toxicity Technical Committee converged as the membership overlapped, but only federal laboratories participated in the EPS committee at that time.

Other organizations conducted aquatic toxicology meetings, such as the long-standing ASTM and SETAC, formed in 1979. Although both meetings attracted larger and wider audiences, their U.S.

focus resulted in ATW being the natural venue to debate issues of Canadian water quality guidelines, toxicity test methods, and effluent regulations.

OECD Duluth Meeting

A separate meeting of ten Organisation for Economic Co-operation and Development (OECD) member countries was hosted by EC and the EPA in Duluth, Minnesota, in 1984 (Environmental Protection Agency and Environment Canada 1984). The goal of the meeting was to provide input for the 1985 OECD "Project on Guidance for the Use of Biological Tests in Decision Making for Water Pollution Assessment and Control". Subgroups were formed to address issues of (i) the application of testing approaches, (ii) use of biological tests in administrative decision making, and (iii) scientific considerations in designing a biological test system. The project was to provide guidance on the analysis and control of toxic effluents that are less costly and more environmentally effective than were approaches at the time.

The 1984 OECD meeting in Duluth published a number of important conclusions that reinforced not only the regulatory direction of Canada and the U.S. in the area of effluent toxicity testing, but that of Europe as well. Some conclusions included:

- Effluent toxicity tests provide valid, cost effective input to protecting the aquatic environment;
- Toxicity test data must be considered together with other chemical and hydraulic information;
- Toxicity tests can be used to establish long-term effluent quality and ambient quality trends;
- Maintaining and improving the skills of testing personnel is important;
- The reproducibility of toxicity tests is comparable to that of chemical tests;
- The use of a standard test species is more important than using resident species for testing;
- Whole effluent tests reflect the interactive effects of chemical components in effluents; and
- A tiered testing scheme should be used for the sake of economy.

Sergy Report / Alliston Workshop / EC Methods Division

While the OECD meeting affirmed an international recognition of the importance of biological testing, EC had already been well along the path of defining the role of ecotoxicological testing (MacGregor and Wells 1984). Initiatives in EC to establish a sound foundation for toxicity testing in industrial effluent regulations were introduced at the 1985 IGATG meeting and led to specific recommendations related to toxicity test procedures for management of EC–EPS (Sergy 1987). From 1985 to 1987, the IGATG committee became an important resource to the EC Toxicity Technical Committee, to the point where IGATG became the prevailing committee, since it included provincial toxicology lab managers and was often chaired by EC representatives.

A parallel activity to define the future for biological testing in Canada was the 1988 stakeholder workshop of industrial representatives, consulting firms, academics, and provincial and federal regulators hosted by EC in Alliston, Ontario. The proceedings of the Alliston workshop (Day et al. 1988) recommended a division of roles for EC (to prepare test protocols and QA/QC requirements), consulting firms (to conduct the regulatory testing) and industry (to report the testing results). The IGATG federal committee members sought funding from within EC–EPS programs shortly after the workshop to establish an external method writing contract, managed by Rick Scroggins, to develop the first set of

standard test methods for rainbow trout and *Daphnia magna* (Environment Canada 1990a, 1990b, 1990c, 1990d). The standardized test methods, prepared by John Sprague and Don McLeay, demonstrated the value of regulatory test protocols and established a viable means by which Canada could maintain sovereignty over its test methods for regulatory testing.

In 1991, EC created the Method Development and Applications Division within EPS. As Chief of this division, Rick Scroggins was charged with continuing to develop standardized aquatic and sediment toxicity test methods. IGATG was already established and functioning in methods review and provided the perfect resource base for the new EC mandate. Throughout the 1990s, Canadian standardized toxicological testing methods were published, including a number of tests measuring sublethal effects to aquatic fish, invertebrates and plants (Environment Canada 1992a, 1992b, 1992c), which drew on the EPA protocols (Weber et al. 1988), EC research methodologies, and the experience developed by many government and private laboratories in the interim.

As the number of EC standardized test methods increased, a wider scope developed and methods came to include soil testing, which prompted a name change in 1999 (Taylor et al. 2013) to Inter-Governmental Ecotoxicological Testing Group (IGETG). IGETG became more inclusive by inviting review of each new method from private sector and academic laboratories across Canada and from experts around the world. An extensive account of the group's accomplishments from its beginnings to 2012 has been detailed by Taylor et al. (2013).

The ATW provided an ideal platform throughout this period for IGATG/IGETG meeting venues and both formal and informal dialogue among sector representatives, drawing on the expanding expertise of federal, provincial, private, and academic testing laboratories. Methods development has been an important contribution to ATW content in terms of platform and poster presentations for more than 20 years.

Environmental Effects Monitoring (EEM)

The EEM regulations (Environment Canada and Department of Fisheries and Oceans 1992) were created to characterize the biological impact of effluent discharges beyond the end of pipe into the receiving environment, another initiative led by Rick Scroggins, Robert Baker, and a group of volunteer EPS technical experts. The regulations required measurement of dioxins, effluent lethal and sublethal toxicity testing, benthic surveys, and fish population assessment (Environment Canada and Department of Fisheries and Oceans 1993). The pulp and paper sector was the first required to conduct effluent quality monitoring for sublethal toxic effects every six months and to report on site-specific receiving environment effects every three years. The mining sector EEM program commenced in late 2002. As the EEM program cycled through its iterations, improvements were made to the various components and some elements were removed, with the pulp and paper sector becoming increasingly involved in the review process. From 1990 to about 2005, the EEM program was another important contributor to the content of ATW through reports, analyses, and opinions developed from that experience.

Technology

The development of computer and Internet technology also had a profound effect on ATW. Affordable desktop computers entered the market in the mid-1980s, reducing the effort of data calculation and publication. The Internet became publicly available by the mid-1990s, enabling the transfer of text, data and graphics files that previously were sent through the mail. The 1997 workshop co-chair, Gordon Craig, registered the domain ATW.CA and created the first versions of the ATW website and an online registration system that simplified logistics and financial workshop administration. The speed of creativity and communication multiplied yearly so that reports and material for presentation exploded and further contributed to the growth in attendance of ATW in the 1990s.

Early Topics, 1975-1977

The initial years of ATW revolved around recurring themes of an early science. Presentations were on laboratory and field assessment methods and how to relate one to the other. Analytical chemistry was rapidly evolving from colourometric to advances in atomic absorption spectrometry, inductive coupled plasma spectrometry, emission spectrometry, gas/liquid chromatography, mass spectrometry and combinations of each. Derivations of water quality criteria were pushing the analytical envelope in that projected effect concentrations were often below analytical detection.

Workshop topics included:

- Acute and sublethal toxicity test methods
- Toxicity tests with fish and invertebrates
- Field/cage toxicity test methods
- Reference toxicants
- Statistical analysis of data
- Industrial and municipal effluent toxicity
- Toxicity of metals and complexes

Maturing Issues, 1978-1987

Presentations became more integrated, pulling together policy, larger geographic issues, and receiving environment impacts. Simplified standardized test methods, such as the rainbow trout lethality test, were being folded into federal regulations and tested in courts. Changing environmental limits required of industrial effluents were pushing advances in effluent treatment from primary to secondary. Effluent toxicity limits were combined with historic and more stringent chemical limits. The challenge of reconciling the acute lethality test with environmental realities was always, "Where are the dead fish?" Ontario and British Columbia were introducing assessments of receiving environments as part of industry's licensing requirements. An environmental profile of habitat quality was being recorded.

Workshop topics included:

- Standard, regulatory acute toxicity test methods
- Smaller, faster, cheaper toxicity tests
- Modifiers of chemical toxicity (e.g., pH, temperature, cations)

- Toxicity of chemical mixtures
- Chemical degradation and changing exposures
- Mutagenicity and carcinogenicity tests
- Fugacity of organics

EEM Years, 1987-2005

Topics transformed from reductionist evaluations to more holistic ones. Multivariate effects were being considered in laboratory and field assessments. Greater focus was being directed to the effect of contaminants and industrial discharges on populations and ecosystems. The receiving environment assessments took on a regulatory form in EEM, first applied to the pulp and paper industry and, later, to the mining industry. The format of the EEM program was being adopted by other industries applying for environmental assessment approvals for greenfield operations. The pulp and paper industry went through three cycles of EEM, making modifications to the program, each time improving the techniques, analysis, and interpretation.

Soil and sediment toxicity test methods also emerged, reaching the shoreline of the aquatic world and beyond. Ecological risk assessment integrated exposure (chemical) analysis, toxicity or dose-response analysis (identifying effect concentrations), and probabilistic estimates. Testing and evaluation methods were becoming refined, more reproducible, and were complemented with other disciplines.

Workshop topics included:

- Standard, regulatory toxicity test methods (e.g., sublethal, marine)
- Toxicity identification evaluations (TIE)
- Toxicity of chemical mixtures
- Structure-activity relationships in toxicity
- Genotoxicity effects
- Endocrine responses and disruption in organisms
- Biochemical tests of effect and exposure
- Cell culture and toxicity testing
- Biomarkers and genomics
- Biotic ligand model of metal toxicity
- Soil ecotoxicity test methods
- Oil sands impacts and reclamation
- Sediment quality guidelines
- EEM study reports (i.e., pulp and paper, mining)
- National interpretation of EEM data
- Fish population responses to pulp mill treated effluent
- Ecological risk assessment methods and reports
- Contaminant modeling in ecosystems (e.g., migration, fate)
- Site-specific water/sediment quality criteria
- Environmental quality guidelines, including national application and harmonization efforts
- Comparison of international studies of receiving environments
- Statistical analysis of test data and large data sets

Current Topics, 2005-Present

The exercise of quantifying the toxicity of various organics and inorganics with newer, more sensitive tests and organisms continued. The library of chemical toxicity data expanded. As a result of national coverage of EEM programs, the quality of effluents such as pulp and paper, sewage and, to some degree, metal mining improved to the point where fish and benthic population effect agents were being teased out by principal component analysis. Pharmaceuticals, personal care products and agricultural products that were previously overshadowed by historic, more concentrated industrial contaminants were now attracting attention. The impact of oil sands development in Alberta on downstream aquatic populations was receiving more investigation and assessment as extraction technologies changed and remediation of treatment ponds and tailings increased in magnitude.

Standardized soil and sediment toxicity tests using invertebrates and plants were being used in ecological risk assessments on a broader scale across the country.

Nanoparticle inclusion into consumer products introduced a contaminant that exhibited unconventional physical and chemical properties. A new paradigm of testing and impact assessment was being developed. Ecotoxicity became the new perspective in environmental assessment.

Workshop topics included:

- Assessment of sewage effluent effects
- Pharmaceutical and personal care product effects
- Immunological responses in organisms
- Principal component analysis of effluents
- Oil sands effects and risk assessments
- Revised water quality criteria protocol
- Character and toxicity of nanoparticles
- Amphibian toxicology
- Synopsis of pulp and paper EEMs
- Metal mining EEM studies
- Fish population responses to mining effluent
- Agricultural stressors on aquatic communities
- Implications of climate change for toxicity impacts
- Arctic studies

ATW Accomplishments

The annual ATW meeting has provided direct personal contact between regulators and industry representatives. Academics have collaborated with federal, provincial, and private research scientists and environmental managers in many studies of mutual interest. Individuals with common interests have published books and papers on the many new technologies, such as the usefulness of microscale toxicity tests or microbiotests (Wells et al. 1997; Blaise and Ferard 2005; Wells and Doe 2014). The meetings have facilitated a better understanding among all sectors and provided a platform to encourage students and introduce them to potential employment prospects.

The tools of aquatic and terrestrial toxicology have been standardized and refined to measure effects on exposed organisms at orders of magnitude lower concentrations than in the days of ten fish in a bucket. The progress has been astounding. Application of these tools has been embedded in large-scale environmental assessments applied to private and public sector industries operating in the country. The discipline of environmental toxicology has advanced so that sensitive population responses to long-term exposure of chemicals, possibly below levels of analytical detection, can now be determined. We now have a very good understanding of our capabilities and know many of the environmental contaminants that can impact biological systems. Our computational knowledge and capability now possibly exceed our ability to measure and detect chemical effects within the milieu of natural ecological variability.

Most important among the development of our skills and knowledge has been establishing ownership over the environmental management tools used in Canada. Canada developed its own water quality objectives, toxicity test and analytical methods that can be refined and modified as national and local needs required. Consequently, Canada became no longer reliant on other countries or multi-national organizations like OECD or the International Organization for Standardization (ISO) to develop sufficiently flexible methods, as was the only option in the 1960s and 1970s. ATW has provided an important and functional forum to address national interests in the protection of aquatic ecosystems in a timely manner over the last 40 years.

Economic Impact of 2008

Management of Canada's environment hit a detour as a consequence of the 2008 global economic downturn. Federal and provincial governments reduced and eliminated programs and staff, industry made adjustments in staffing, and the consulting sector experienced mergers and acquisitions. Many in the "baby boomer" cohort of senior researchers and managers who would have retired out by 2013 left a few years earlier due to early retirements or terminations after the recession hit.

The federal Bill C-38 revised a number of environmental statutes including the *Fisheries Act, Canadian Environmental Assessment Act, Canadian Environmental Protection Act, 1999* and *Navigable Waters Protection Act*. These statutory changes reduced the obligations of industry to monitor and protect environmental quality and health. Regulatory changes also provided $160 million in federal cost savings through program reductions (The Toronto Star 2012).

Employment in public and private environmental programs decreased as a result of the 2008 economic downturn. Since attrition was one of the employment reduction strategies, the number of senior experienced scientists and managers declined and, in turn, the loss of those they attracted to meetings accentuated the reduction in ATW attendance numbers in 2011.

Future Direction for ATW

In order to keep the focus and direction of workshop themes aligned with current needs, the ATW Board of Directors created an Advisory Committee in 2013 to identify pressing local, national and global environmental issues to assist local organizing committees in their planning. Committee members were invited from past and current organizing committees, representing industry, academia,

and government sectors. The Advisory Committee was charged to recommend themes, specific topics and guest speakers for future workshops, so as to attract broader participation and keep the program fresh and relevant to current issues important in Canada.

Setting Ecosystem Objectives

The biggest challenge before environmental managers today is to alter the style of environmental management and incorporate a proactive approach. The last forty years have been largely reactive except, to some degree, in the development of water quality criteria. However, the criteria largely deal with single toxicants and seldom address combined and cumulative impacts of other stressors.

While environmental assessments of various industrial projects have addressed the issue of cumulative effects and multiple stressor interaction, they have been largely hypothetical models based on simple assumptions. A good start, but more work is required to ground truth relationships.

The next phase of managing, protecting, and preserving our environmental quality will be led by the ecologists. We need to define and quantify healthy sustainable populations and communities of organisms for a given geographical area.

An early example of ecosystem management (Ryder and Edwards 1985) was developed in the 1980s by the Aquatic Ecosystems Objectives Committee of the International Joint Commission (IJC) at a time when the required tools were in their infancy and jurisdictional will was weak. The IJC committee developed four separate ecosystem objectives for Lake Trout, Walleye, the Bald Eagle, and *Pontoporeia* to be applied to the appropriate Great Lakes. The health of a top predator was predicated on the health of the sustaining ecosystem, and the health of a lower trophic-level population was a prerequisite for healthy, higher foraging populations. Health indicators included reproductive successes, growth rates, presence of endogenous contaminants, feeding habits, habitat quality, and others.

The EEM fish population reproductive health assessment also fits this paradigm and has been studied extensively by Kelly Munkittrick from University of New Brunswick and his many colleagues (Munkittrick et al. 2010). The EEM fish community assessment represented a refinement of approaches developed by Harold Harvey of the University of Toronto (Beamish and Harvey 1972) and the study of acid rain effects on fish populations.

The Bay of Fundy Ecosystem Partnership, formed in 1997, engages a range of public, private, academic sectors and community groups across international borders and focuses on managing the Bay as a whole ecosystem (Vickers et al. 2012). Such a holistic approach to environmental protection and assessment needs further development and adoption. Once the principal physical, chemical and biological needs of the population are defined and refined, specific, quantifiable ecological targets should be identified. Determining why those ecological targets are not being met will enable specific levels of performance to be identified for industries, sectors, and urban communities. This holistic approach can build on the joint interests of regulators and researchers in the private and public sectors that began during the EEM program in the 1990s.

Communication networks, proven analytical and biological tools, and a well-developed computational capability provide the tools to advance assessment techniques. Jurisdictional will needs to be motivated but it also needs to be informed. Community groups, together with a trained, educated retirement force, can play an important role in the political arena to capture the hearts and minds of our elected representatives.

ATW has a continuing role to report on current conditions and promote the integration of disciplines and skills in addressing national and, by example, international needs for a healthier environment.

Sponsors and Supporters

ATW has stayed vibrant through the enthusiasm of organizing committees and the leadership of the Chairs, who represent the foundation of its longevity and success.

ATW has benefited from the long-term support of DFO and EC, both through the provision of staff time and direct funding. All the ATW continuity Chairs have been from these two organizations.

ATW has also received monetary and staff resources from provincial governments, universities, consulting companies, and resource, manufacturing and service industries when the annual meeting has arrived in their respective regions. Everyone has participated and worked together to make each workshop a success. The logos of many of ATW's sponsors (Figure 5), some of which have been acquired or merged, provide an indication of the broad base of support over the years.

Figure 5. Many of the ATW sponsors over 40 years

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Appendix 1. ATW Chairs and organizing committee members since 1974

Year	Chairs	Organizing Committee
1974 Winnipeg	John Loch & John Davis	None identified
1975 Toronto	Gordon Craig	C. Inniss, D. Wells, M. Whittle, K. Suns, J. Reinke, T. Lagan, W. Baksi, P. Hunter, J. Fitzsimons, S. Norman
1976 Halifax	Ed Pessah & Peter Wells	R. Parker, G. Westlake
1977 Vancouver	John Davis	G. Greer, I. Birtwell, G. Vigers, R. Hoos, M. Waldichuk, B. Smythe, N. Holeman, I. Shand, P. Vroom, S. Nelles, D. McLeay
1978 Hamilton	Peter Hodson	P. Wong, A. Niimi, V. Cairns, U. Borgman, K. Kaiser, B. Blunt, O. Kramar, C. Loveridge, L. Luxon, D. Spry
1979 Winnipeg	Jack Klaverkamp & Lyle Lockhart	A. Blouw, R. Clarke, S. Leonhard, D. Malley, M. McLean, E. Scherer, M. Yakimischak
1980 Montreal	Norman Bermingham & Gérard Leduc	C. Blaise, M. Speyer, P. Couture, G. Joubert, B. Hummel, R. Van Coillie, C. Thellen, M. Lebel, L. Sprags
1981 Guelph	Gordon Craig & Keith Solomon	F. Beamish, K. Day, J. Hilton, P. Hodson, D. Holdway, N. Hutchinson, N. Kaushik, D. Rogers, J. Sprague, C. Wren
1982 Edmonton	Bill Lake	A. Hrynyk, A. Beckett, W. Mackay, M. Strosher, B. Munson, B. Hammond, J. Kotler, J. Retallack
1983 Halifax	Richard Addison & Peter Wells	M. Hutcheson, R. Parker, J. Uthe, G. Westlake
1984 Vancouver	Glen Geen	I. Birtwell, K. Hall, A. McCarter, D. McLeay, R. Morley, M. Roch, R. Watts
1985 Thunder Bay	George Ozburn & Howard McComick	A. Smith, OMOE staff, Lakehead University staff
1986 Moncton	J. Lakshminarayana	T. Pollock, R. Cote, M. Gilbertson, H. McCormick, J. Uthe, P. Wells, V. Zitko, P. LeBlanc, C. Morry, R. Parker, M. Butler, T. Clair, D. Eidt, P. Pearce, D. Besner, P. Maltais, Y. Poussart, R. South, R. Addison
1987 Toronto	Gary Westlake	S. Abernethy, A. Niimi, K. Solomon, P. Stokes
1988 Montreal	Raymond Van Coillie	A. Champoux, P. Anderson, J. Boulva, J. Brodeur, R. Brouzes, J. Cerf, G. Chevalier, R. McLean, P. Ross, H. St-Martin, C. Blaise, D. Brouard, R. Coté, P. Courure. J. de la Noüe, P. Lundahl, C. Marengo, J. Massicotte-Pellerin, B. Pinel-Alloul, Y. Roy, H. Sloterdijk, M. Speyer, C. Thellen, B. Trotier, Y. Vigneault, N. Birmingham, M. Sinotte

Year	Chairs	Organizing Committee
1990 Vancouver	Peter Chapman	K. Hall, L. Harding, D. McLeay, M. Nassichuk, S. Yee, W. Knapp, E. Power, W. Schwartz, L. Reid
1991 Ottawa	Margaret Taylor	L. Bendell-Young, J. Jensen, K. Lloyd, C. Macdonald, F. Pick, R. Pierce, R. Scroggins, R. Shearer, C. Wyndham
1992 Edmonton	Earl Baddaloo	A. Kozlowski, W. Mackay, M. Mackinnon, J. Moore, S. Ramamorthy, P. Humphrey
1993 Quebec City	Raymond Van Coillie	C. Thellen, P. Campbell, H. Pagé, L. Hare, L. Martel, J. Piuze, R. Schetangne, R. Siron, B. Pinel-Alloul, P. Anderson, D. Brouard, J. Labrie, N. Bermingham, Y. Roy, Y. Bois, P. Lundhal, P. Riebel
1994 Sarnia	Scott Munro & Ted Kierstead	K. Solomon, G. Craig, G. Westlake, M. Lines, A. Niimi, S. Thornley, J. Parrott, C. Facca, K. Hall
1995 St. Andrews	Kats Haya	J. Arsenault, L. Buridge, S. Courtenay, W. Fairchild, B. Waiwood, L. White, V. Zitko, H. Akagi, B. Best, M. Bender, B. Hatt, M. Saunders, M. Lyons, K. MacKeigan, B. Neilson
1996 Calgary	Stella Swanson & D. Treissman	M. Brown, L. Clader, R. Compton, C. Gervais, S. Goudey, L. Linton, J. Nagendran, T. Nason, T. Nerberg, R. Robinson, S. Silva, B. Steinback
1997 Niagara Falls	Joanne Parrott & Gordon Craig	P. Dehn, G. Dixon, D. Hart, S. Humphrey, K. Holtze, P. McKee, T. Moran, A. Niimi, L. Novak, D. Rokosh, C. Wren, M. Burley, L. Gysbers, J. Smits
1998 Quebec City	Raymond Van Coillie	C. Thallen, G. Van Coillie, H. Pagé, C. Blaise, J. Bureau, R. Chassé, C. Couillard, I. Guay, L. Hare, C. Langios, L. Martel, L. Parent, S. Pauwels, R. Prairie, R. Schetagne, G. Sunahara, P. Wells, J. Pellerin, J. Karau, J. Labrie, Y. Bois, C. Côté, I. Cloutier, G. Grenon, G. Craig, L. Trudel, L. Veilleux
1999 Edmonton	Earl Baddaloo	D. Birkholz, M. Brown, M. Clendenan, S. Goudey, M. Mah-Paulson, A. Verbeek, M. Fairbairn
2000 St. John's	Kathy Penny & Kim Coady	M. Murdock, R. Parker, D. Snow, P. Jackman, E. Tracy, S. Whiteway, P. Orr, E. Luiker
2001 Winnipeg	Michael McKernan Brian Wilkes	B. Bayer, G. Craig, D. Harron, K. Mathers, M-A. Phare, G. Stern, D. Williamson
2002 Whistler	Curtis Eickhoff & Graham van Aggelen	J. Boyd, D. Bright, J. Bruno, K. Hall, P. Keen, C. Kennedy, K. Kinnee, P. Lim, C. Lowe, A. Niimi, M. Paine, B. Raymond, S. Yee
2003 Ottawa	Kathleen Hedley	M. Bombardier, S. Desjardins, G. Gilron, V. Hodge, G. Kaminski, B. Kilgour, J. McGeer, T. Moon, J. Nadeau, C. Portt, G. Rawn, S. Roe, M. Schwartz, D. Spry, G. Tétreault, V. Trudeau, S. Winch

Year	Chairs	Organizing Committee
2004 Charlottetown	Les Burridge	W. Ernst, W. Fairchild, K. Haya, J. Hellou, R. Allen Jarvis, B. Kilgor, K. Lee, M. Murdock, R. Parker, S. Smith-Gray, S. Steller, K. Teather
2005 Waterloo	George Dixon & Scott Munro	G. Craig, P. Dehn, A. Farwell, J. Green, L. Lee, M. McMaster, T. Moran, W. Norwood, C. Portt, J. Schroeder, J. Sherry, G. Stephenson, G. Tétreault
2006 Jasper	Barry Munson	N. Ali, J. Ferone, J. Froese, P. Siwik, M. Dube, V. St. Louis, L. Noton, C. Renzenbrink, M. Foster, L. Wood, D. Beauparlant, S. Boss, T. Steiglitz, C. Maxwell, A. Petersen, C. Rickwood, A. Squires, R. Beaulieu, M. Gray, K. Fraser
2007 Halifax	Karen Kidd & Rosalie Allen Jarvis	K. Doe, K. Haya, G. Benoy, C. Burnett, S. Courtenay, C. Hedley, J. Hellou, T. Jardine, C. Moore, R. Mroz, K. Munkittrick, M. Murdock, L. Rutherford, V. Soehl, M. van den Heuvel, P. Wells
2008 Saskatoon	Karsten Liber	A. Brown, C. Burnett, M. Dubé, D. Duro, J. Geisy, S. Geisy, N. Glozier, K. Hancock, M. Hecker, K. Himbeault, D. Janz, P. Jones, P. Krone, S. Niyogi, M. Pietrock, J. Price, S. Sedgewick, A. Squire, L. Weber
2009 La Malbaie	Louis Martel & Michel Fournier	P. Benoît, D. Berryman, T. Bosker, M. Bouchard, O. Bouchet, A. Boullimant, P. Campbell, L. Champoux, C. Couillard, N. Dassylva, F. Gagné, C. Gagnon, I. Guay, P. Juneau, E. Lacroix, M. Lebeuf, L. Parent, R. Patenaude, J. Pelllerin, P.Y. Robidoux, G. Tiffault-Bouchet, B. Vigneault
2010 Toronto	Tim Fletcher & Douglas Holdway	T. Watson-Leung, P. Welsh, N. Feisthauer, D. McLatchy, G. van der Kraak, A. Bartlett, W. Norwood, C. Metcalfe, M. Dutton, B. Kilgour, L. Novak, M. Rendas, D. Poirier, J. Anderson, Y. Gopalapillai, L. McCarthy, J. Schroeder, M. Nowierski, D. Simmons, J. Guchardi
2011 Winnipeg	Karen Mathers & David Heubert	J. Anderson, B. Glowacka, M. Hanson, J. Heibert, H. Loomer, M. McKernan, S. Veroukis, C. Wong
2012 Sun Peaks	Joanne Harkness & Graham van Aggelen	J. Bruno, C. Eickhoff, C. Helbing, C. Kennedy, P. Kickham, H. Loomer, C. Lowe, J. Muscatello, R. Prosser, G. Schroeder, B. Yates
2013 Moncton	Paula Jackman & Les Burridge	M. Murdock, R. Morais, D. Daoud, S.E. Maher, J. Van Geest, R. Prosser, J. Challis, G. Schroeder, L. Miller
2014 Ottawa	Lisa Taylor & Carrie Rickwood	S. Agius, B. Cameron, J. Challis, M. Desforges, C. Eickhoff, G. Gilron, Y. Gopalapillai, S. MacLeod, A. Muhametsafina, L. Novak, M. Rendas, S. Siwik, G. Schroeder, P. Siwik, L. Tupper-Ring, L. Van der Vliet
2015 Saskatoon	Karsten Liber & David Janz	Being solicited

Acronyms

ASTM	American Society for Testing and Materials
ATW	Aquatic Toxicity Workshop
APHA	American Public Health Association
CCME	Canadian Council of Ministers of the Environment
DFO	Department of Fisheries and Oceans
EC	Environment Canada
EEM	Environmental Effects Monitoring
EPA	United States Environmental Protection Agency
EPS	Environmental Protection Service (Environment Canada)
IGATG	Inter-Governmental Aquatic Toxicity Group
IGETG	Inter-Governmental Aquatic Ecotoxicity Group
IJC	International Joint Commission
ISO	International Organization of Standardization
NAS	National Academy of Sciences
OECD	Organisation for Economic Co-operation and Development
OMOE	Ontario Ministry of Environment
OMOEE	Ontario Ministry of Environment and Energy
OWRC	Ontario Water Resources Commission
SETAC	Society of Environmental Toxicology and Chemistry
SOP	Standard Operating Procedure

Acknowledgments

Paula Jackman and Les Burridge, Co-Chairs of the 2013 workshop, invited me to prepare a reflection of 40 years of ATW for this workshop; otherwise, it probably would not have happened. Most statistical material came from the past proceedings, which were digitized by Les Burridge when he was Continuity Chair of the ATW Board; they are posted on the ATW website (Aquatic Toxicity Workshop 2014). Rick Scoggins, who has been involved in methods development most of his career, clarified many of the early events of IGATG and the EEM program. Peter Wells, a champion of science history all his career, provided copies of past reports and papers, recollections of his own, reviewed the drafts and cheered on the progress of each. Rosalie Allen Jarvis provided final edits with my gratitude. Errors and omissions are my own. Thanks to all above and to those listed in the Appendix who have made each of the past workshops a national success.

Dr. Richard C. Playle Awards for Outstanding Theses in Aquatic Toxicology

For the first time, there were two winners of the master's-level award in 2013. The winners of the Playle Award for Outstanding MSc Thesis in Aquatic Toxicology were Amanda Carew from the University of Victoria and Emily-Jane Costa from Wilfrid Laurier University. There was no winner of the bachelor's-level award in 2013.

The sublethal effects of nanosilver on thyroid hormone-dependent frog metamorphosis (PL)

A. Carew[1], C. Helbing[1]
[1]University of Victoria

Nanoparticles are engineered in the nanoscale (<100nm) to have unique physico-chemical properties from their bulk counterparts. Nanosilver (nAg) is the most prevalent nanoparticle in consumer products due to its strong antimicrobial action. While nAg toxicity at high concentrations has been well described, its sublethal effects at or below regulatory guidelines are relatively unknown. Amphibian metamorphosis is mediated by thyroid hormone (TH), and our initial studies indicated that low concentrations of nAg disrupted TH-dependent responses in precociously induced premetamorphic bullfrog (*Rana catesbeiana*) tadpoles. The study examined the effects of low, environmentally relevant nAg concentrations on naturally metamorphosing *Xenopus laevis* tadpoles in two 28-day chronic exposures beginning with pre- and prometamorphic stages, respectively. nAg was found to significantly bioaccumulate in tadpoles after 28 days. While nAg did not alter metamorphic timing, it increased hindlimb length during early premetamorphosis and in post-metamorphic juvenile tadpoles. Using MAGEX microarray and QPCR transcript analyses, seven markers of nAg exposure were discovered and validated, five of which showed nAg-induced disruption of their TH-response. The increased mRNA abundance of two peroxidase genes suggests that nAg could generate reactive oxygen species (ROS) even at low environmental concentrations. Furthermore, differential responsiveness to nAg was observed between developmental stages. Therefore, low concentrations of nAg had endocrine disrupting effects at both the physiological and molecular level, indicating that regulatory guidelines for silver may need revision.

The acute and chronic toxicity of three forms of nanoparticle silver and silver nitrate to *Daphnia pulex*: Is toxicity related to particle dissolution? (PL)

E.-J. Costa[1], J. C. McGeer[2]
[1]Golder Associates Ltd., [2]Wilfrid Laurier University

This research aims to understand the acute and chronic uptake and toxicity of nanoparticle silver (nAg) to *Daphnia pulex* through comparisons to ionic silver (Ag^+). A three-step approach considered toxicity, solution characterization, and physiological effects to determine if nAg toxicity is due to individual particles or particle dissolution to Ag^+. Acute and chronic toxicity tests were standard 48-hour and 21-day exposures, respectively, to determine 25% (EC_{25}) and 50% effect concentrations (EC_{50}). Using filtration and dissolution techniques, solutions at these concentrations were measured for size fractions (e.g., Ag content in < 450nm, <100nm, < 10nm and < 1nm fractions) and assessed for the relative content of nAg and Ag^+ associated with effects. The size fractionation and dissolution assessments were done on three solutions: water only, water + neonates (acute simulation), and water + adult *Daphnia* + food (chronic simulation). To assess physiological effects, 6-day-old *Daphnia* were exposed to the chronic EC_{25} ($0.4\ \mu g \cdot L^{-1}$ Ag) to measure short-term (3-hour) and long-term (48-hour) bioaccumulation, as well as ^{22}Na influx (to determine if nAg disrupts Na balance). Acute toxicity was comparable for each form of Ag tested (EC_{50} of ~$0.8\ \mu g \cdot L^{-1}$) but chronic toxicity differed. Exposure solution characterization revealed that dissolution of nanoparticles to form ionic silver in solution does not play a significant role in nAg toxicity, and characterization of water only, acute-simulated, and chronic-simulated solutions can reveal different particle size distributions. Together, these results show that nAg toxicity cannot be solely related to particle dissolution to Ag^+.

Sublethal Endpoints

Opportunistic disease and immune effects in fish exposed to oil sands-affected waters (PL)

N. S. Hogan[1], K. Thorpe[2], R. Van Aerle[3], M. R. van den Heuvel[4]
[1]University of Saskatchewan, [2]University of Portsmouth, [3]University of Exeter, [4]University of Prince Edward Island

In studies spanning more than a decade, yellow perch (*Perca flavescens*) were exposed to oil sands-affected waters in an effort to determine their impact on fish health. A particular focus was on the ability of these waters to affect immune function and increase disease incidence in exposed fish. The experimental program involved stocking studies using yellow perch conducted during 1995-1997 and 2008-2010. This *in situ* research was conducted with waters from three primary sites: Mildred Lake, a reservoir receiving only Athabasca River water, used as the reference site; South Bison Pond, a drainage basin that has received unextracted oil sands-contaminated overburden; and Demonstration Pond, a constructed pond containing oil sands mature fine tailings capped with fresh water. In both sets of studies, two disease pathologies were observed in yellow perch: fin erosion caused by an as yet unidentified pathogen, and lymphocystis, which was confirmed using a diagnostic real-time PCR method. In early studies (1995-1997), both pathologies were most prevalent in the South Bison Pond; however, in the recent studies (2008-2010), disease was substantially more prevalent in the Demonstration Pond. In order to evaluate the transcriptomic pattern of immune dysfunction, mRNA extracted from the head kidney of exposed and unexposed perch was evaluated using ILLUMINA sequencing. Transcriptomic analysis revealed upregulation of B-cell receptor, antimicrobial peptides and MHC I receptor while downregulation of C-type lectins, immunoglobulins (Ig), killer cell Ig receptor and components of the complement system were observed. Due to the complexity of the exposure environment, precise causative agents are not yet known, and results are discussed in the context of water chemistry changes over the decade-and-a-half of study.

Cellular-level responses of rainbow trout (*Oncorhynchus mykiss*) leukocytes to benzo[a]pyrene (PL)

L. Phalen[1], B. Koellner[2], M. R. van den Heuvel[1]
[1]University of Prince Edward Island, [2]Friedrich-Loeffler-Institut

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that may pose a threat to fish populations. Observations of immune impairment following exposure to this family of chemicals in field and laboratory studies incite interest in investigating toxicity to isolated immune cells. The aim of this study is to establish an *in vitro* model for investigating effects of benzo[a]pyrene (BaP), a model PAH, on rainbow trout (*Oncorhynchus mykiss*) immune cells. Leukocytes were isolated by

density gradient and primary culture parameters were established. Cells were then exposed to one of five doses of BaP, and cell viability was determined at 24, 48 and 72 hours. Propidium iodide ("dead stain") and fluorescein diacetate ("live stain") staining procedures were optimized for these cells and were analyzed using fluorescence-assisted cell sorting. In the absence of light, no cytotoxicity was observed at any time point or dose. In the presence of light, however, a decrease in live staining of 60% was observed after 24 hours of exposure to 5 μM of BaP. This indicates a response to metabolized BaP and an inability to metabolize BaP sufficiently within tested time frames. An extracellular metabolism system using hepatic microsomes from β-naphthoflavone-induced rainbow trout was evaluated for the production of toxic metabolites. The formation of metabolites was confirmed using HPLC with fluorescence detection. Despite the evidence for the presence of CYP1A in leukocytes, cells appear to be resistant to BaP toxic effects due to a limited ability to produce oxidative metabolites.

Determining the mode of toxic action: Do all phthalates go to same party? (PL)

L. Tupper-Ring[1], F. A. P. C. Gobas[1]
[1]Simon Fraser University

Phthalates are heavily used as plasticizers and are now ubiquitous in the environment. Individually, phthalates do not pose a serious environmental health risk, yet their cumulative risk remains unknown. The goal of this research is to identify the mode of toxic action for phthalates as the initial step in determining the cumulative environmental risk of phthalates. The scientific community believes phthalates have a non-polar narcosis mode of toxic action, yet this still remains unknown. To address this uncertainty, an activity-based approach will be applied on internal acute toxicity data for phthalates. This approach will not only eliminate toxicity endpoints above their solubility limit, but also presents all the toxicity endpoints using one common unit, which allows for better analysis between the different internal toxicity endpoints. Completed results will be presented. The results of this analysis could help suggest an alternative method, using existing data, for identifying the mode of toxic action for other chemicals, and complete the initial step in identifying the cumulative risk of phthalates.

Rapid assessment of the genotoxic potential of environmental contaminants using fish cell lines (PL)

L. Lee[1], M. Trento[1], J. Alexander[1], F. Rojas[1], A. Chun[2]
[1]University of the Fraser Valley, [2]Wilfrid Laurier University

The aquatic environment is usually the final sink for environmental contaminants, including genotoxicants. Piscinids encompass the largest vertebrate group and are an important population in aquatic environments that are susceptible to genotoxicants. These effects are difficult to assess *in vivo,* however, because expensive aquatic facilities and complex, time-consuming procedures would be needed. Cell lines have been very useful for evaluating genotoxicant effects on mammals, but their use for genotoxic assessment of aquatic organisms has been limited; thus, fish cell lines could be useful. Comparatively few studies have been reported with fish cell lines for the evaluation of genotoxicants

and very few reports provide detailed procedures. In this presentation, we provide a step-by-step process in the use of fish cell lines for evaluating genotoxic potential of contaminants, using "comet" and "micronuclei" assays and providing examples with model ecotoxicants, including naphthenic acids from oil sands process waters.

The aryl hydrocarbon receptor pathway of sturgeon: Evolutionary and ecotoxicological implications to dioxin sensitivity in fishes (PL)

J. Doering[1], S. Wiseman[1], S. Beitel[1], J. Giesy[1], M. Hecker[1]
[1]University of Saskatchewan

Sturgeons are ancient species of fishes with recognizable fossils dating back at least 65 million years. Today, many sturgeon species around the world are endangered, which has rendered these fishes of great interest in context with the ecological risk assessment of anthropogenic stressors. However, current risk assessment attempts are hampered by a lack of knowledge about the sensitivity of sturgeons or other ancient species to toxicants of concern. One such class of compounds is dioxin-like compounds (DLCs), which are known to cause a variety of adverse effects in fishes. All adverse effects of DLCs are believed to be mediated by activation of the aryl hydrocarbon receptor (AhR). Although sturgeons have been shown to be highly responsive to DLC exposure, nothing is known about the AhR pathway in sturgeons or other ancient species of fishes. In an attempt to characterize the AhR pathway of sturgeons, we identified at least two AhRs expressed in white sturgeon (*Acipenser transmontanus*) and lake sturgeon (*A. fulvescens*). One AhR shares greatest amino acid identity with the AhR2 in other fishes, while the other shares greatest amino acid identity with the AhR1 found in birds and other tetrapods. Both AhRs have greatest expression in livers and hearts of sturgeons, which are major target organs for dioxin-like effects, and were up-regulated following exposure to a model DLC. The toxicological significance of sturgeons expressing an AhR with great similarity to the AhR1 of birds is currently unclear. To date, the risk assessment of DLCs in fishes uses toxic equivalency factors (TEFs) based largely on studies with salmonids; however, the AhR pathway of sturgeons appears to differ significantly from that of salmonids or other teleost fishes, which might impair proper protection of sturgeons based on current TEFs. Additionally, although fishes are relatively insensitive to mono-ortho-PCBs and some other DLCs compared to birds and mammals, it is hypothesized that sturgeons might be significantly more sensitive to these compounds than other fishes due to their avian-like AhR. Further knowledge of the AhR pathway of sturgeons might reveal interesting structural and functional implications for fishes, as the sensitivity of birds can be predicted based on subtle amino acid differences in the sequence of the AhR1 ligand binding domain. However, such a predictive relationship has not yet been established in fishes, largely due to a number of constraints in the complexity of the AhR pathway of most teleosts.

Characterizing gene networks in rainbow darter (*Etheostoma caeruleum*) to better understand the mechanisms underlying intersex condition in fish exposed to municipal effluents (PL)

P. Bahamonde Cárdenas[1], M. E. McMaster[2], M. R. Servos[3], C. J. Martyniuk[1], K. R. Munkittrick[1]
[1]University of New Brunswick, [2]Environment Canada, [3]University of Waterloo

The rainbow darter (*Etheostoma caeruleum*) is a small benthic fish found in North America. This species is sensitive to sewage effluent in the environment, showing the presence of intersex in up to 80% of males in near-field areas in the Grand River, Ontario. This study approached the issue by developing a customized oligonucleotide microarray (4x180K) with next generation sequencing (454 Roche) to evaluate molecular responses in the gonad. Gene expression analysis showed an over-expression of genes associated with oogenesis in intersex fish, and a reduction of genes associated with spermatid development. In females exposed to effluent, cell processes related to hatching and ovulation were down-regulated, and genes involved in immune responses were increased. In the non-intersex males exposed to effluent, cell processes such as sperm cell adhesion were depressed in expression. Heat shock proteins (HSP) were significantly affected in males exposed to effluent; however, these were not affected in intersex effluent-exposed fish. A knowledge gap in our understanding of intersex is whether there are individuals more sensitive to developing the condition, and if so, whether there is a molecular basis for this sensitivity. Our research is focused on addressing this question. The arrays allowed identification of unique patterns of gene responses that were further evaluated using real-time PCR to develop tools for detecting the potential for intersex. Genes involved in sexual differentiation (sox9b, foxl2 and dmrt1) and reproduction (esr1, erb, ar, vtg, aromatase and cyp11a) were evaluated in males, females, and intersex individuals. Consistent with the intersex condition, many transcripts showed an intermediate expression level in intersex males when compared to phenotypic males and females. Furthermore, a suite of qPCR probes will be developed that includes genes that discriminate pollutant-exposed males without intersex and those males with intersex.

Fish reproductive effects of select pharmaceuticals (PL)

L. Beyger[1], J. Guchardi[1], D. A. Holdway[1]
[1]University of Ontario Institute of Technology

Non-steroidal anti-inflammatory drugs, along with estradiol, are the most frequently detected pharmaceuticals in the environment due to their high level of use and ease of access (Santos et al. 2010) and more knowledge regarding the impacts of environmentally relevant concentrations of such pharmaceuticals on fish reproduction is required. A study using Florida flagfish (*Jordanella floridae*) breeding harems was conducted to monitor the reproductive effects of exposure to ibuprofen (0.1 $\mu g \cdot L^{-1}$), naproxen (0.1 $\mu g \cdot L^{-1}$), and 17α-ethynylestradiol (10 $ng \cdot L^{-1}$) alone, and in a mixture. Flagfish were placed into breeding harems with 2 males and 4 females per treatment in triplicate alongside controls. The experiment was conducted in 70 L aquaria with pre-exposure (21-day), exposure (21-day) and post-exposure (7-day) phases during which the reproductive endpoints were monitored. Reproductive

endpoints of fertilization, hatchability, and egg production were of particular interest. Fertilization was significantly reduced ($p \leq 0.05$) in flagfish exposed to 0.1 $\mu g \cdot L^{-1}$ naproxen and to 10 $ng \cdot L^{-1}$ 17α-ethynylestradiol. Hatchability and egg production were not affected following exposure to the pharmaceuticals. While reproductive effects are well known for 17α-ethynylestradiol, to our knowledge, the effects of naproxen on fertilization at the environmentally relevant concentration of 0.1 $\mu g \cdot L^{-1}$ has not been reported previously. This finding indicates that more research is necessary into the potential reproductive effects of environmentally realistic exposures of fish and other aquatic organisms to naproxen.

Challenges and opportunities in spatiotemporal analyses of fish mercury databases and literature data (PL)

L. Campbell[1], D. Depew[2], S. Bhavsar[3], N. Burgess[2], R. Lavoie[4], M. Chumchal[5], R. Drenner[5], K. A. Kidd[6], E. Delong[7]

[1]Saint Mary's University, [2]Environment Canada, [3]Ontario Ministry of Environment, [4]Queen's University, [5]Texas Christian University, [6]University of New Brunswick, [7]Borealis Geomatics

Mercury monitoring of freshwater sportfish has been conducted by a number of governments around the world since the 1970s. As a result, there are now extensive spatiotemporal databases on mercury in sportfish available for analyses, in addition to a fast-growing body of mercury biomagnification literature. Our research group has compiled a global database on mercury biomagnification trends, worked with the Province of Ontario's Sportfish Contaminants Monitoring Database, and has developed a large-scale National Fish Mercury Database by compiling multiple databases across Canada. We discuss the challenges inherent in developing and analyzing those large databases and our approaches to resolving those challenges. We also report significant trends in sportfish mercury in Ontario, across Canada, as well as biomagnification trends around the world. The use of multiple databases for spatiotemporal trend analyses, ecological risk assessment, and the effectiveness of past, current, and future mercury regulation will be discussed.

Validating the use of molecular tools in fish-based Environmental Effects Monitoring programs (PO)

C. Nzirorera[1], L. Miller[2], M. Doyle[3], C. J. Martyniuk[3], K. R. Munkittrick[3]

[1]University of New Brunswick, [2]British Columbia Ministry of Environment, [3]University of New Brunswick / Canadian River Institute

Although there is debate in the literature about their applicability to fish-based Environmental Effects Monitoring programs, molecular tools have the potential to detect changes in organisms before they are observed at higher-level endpoints in individuals or population level. To determine if molecular tools can be confounded by the acute stress of field sampling procedures, expression levels of genes involved in oxidative and cellular stress were measured using quantitative real-time PCR. Male slimy

sculpin (n = 10 per treatment) were collected from the Kennebecasis River, a reference site near Calamingo Brook Bridge, New Brunswick. Fish were collected by backpack electro-shocker, and held in aerated buckets for 0, 30, 60 and 120 minutes before sampling. Histology showed males were spawning, and the age range (2-4 years, with an average of 2.75 years) was determined using otoliths. The mRNA levels of glutathione reductase (gr), superoxide dismutase (sod), catalase (cat), glycogen synthase (gys), and heat shock protein 90 (hsp90) were measured in liver. No significant differences among treatment groups (ANOVA, $p > 0.05$) were observed for any of the genes investigated. The non-significant levels of gene expression among treatment groups suggest that select molecular biomarkers are robust enough not to be affected by acute stress caused by holding time.

Individual gene variability and correlates in the fathead minnow ovary: Implications for critical effect size and experimental power (PO)

A. Cowie[1], J. R. Loughery[1], R. K. Wood[1], Y. Z. Chishti[1], C. J. Martyniuk[1]
[1]University of New Brunswick

Gene expression profiling in fish physiology and ecotoxicology studies is becoming more prevalent; however, studies characterizing variability in mRNA levels in individual fish tissues are lacking. We assessed gene variability in steroidogenic genes and sex-steroid receptors to shed light on how gene expression patterns relate to higher-level endpoints commonly assessed in environmental monitoring programs. We measured transcripts involved in receptor signaling and steroidogenesis in the ovary of fathead minnow (*Pimephales promelas*) (n = 15). We correlated mRNA levels to endpoints such as gonadosomatic index, condition factor, oocytes stage, and ovarian steroid production (E2 and T). Steroid receptors such as esrbb and membrane progestin receptors (beta and gamma) showed high variability in the fathead minnow ovary while pgr, ar, and esrba showed comparatively low variability. Steroidogenic enzymes that included srd5a2 and srd5a3 showed high variability in the fathead minnow ovary whereas hsd3b and srd5a1 showed comparatively low variability. Both cyp19a1 and hsd17b were significantly correlated to T:E2 ratio. The mRNA levels of cyp19a1 were significantly positively correlated to both ar and pgr mRNA levels, and star mRNA levels were significantly positively correlated to esr1, esr2b, and ar mRNA levels. Thus, ar signalling may play a prominent role in regulating the transcription of key rate-limiting steps in steroidogenesis (i.e., star and cyp19a1). Using forward regression and least square modeling, GSI, T, and cyp19a1 explained >80% of the variability in E2 production. Small-bodied fish are increasingly used in both laboratory and field studies to characterize biological responses to environmental contaminants and biotic stressors, and these data can help guide researchers in molecular-based assessments (i.e., critical effect sizes and experimental power), thereby determining what is a biologically or statistically significant change.

Gene expression changes in wild yellow perch (*Perca flavescens*) brain due to methylmercury exposure (PO)

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Kejimkujik National Park, Nova Scotia, is a biological mercury (Hg) hotspot, and yellow perch (*Perca flavescens*) in the lakes are known to have Hg concentrations in tissues that exceed safe limits for fish and fish-eating wildlife. However, the mode of action for toxicity of the organic and dominant form of total Hg (THg), methyl Hg (MeHg), is not fully known, and some field studies have found links between Hg exposure and the reproductive and general health of fishes. In this study, gene expression was used to determine if yellow perch from Kejimkujik National Park are being affected by MeHg at the molecular level, specifically in the brain. Protein-coding genes targeting oxidative stress (superoxide dismutase, heat shock protein, and glutathione-S-transferase) and mechanisms of MeHg toxicity (protein disulfide isomerase) were analyzed in 39 female perch from five lakes using real-time PCR. Fish were sampled in September 2010, and muscle and brain tissues were frozen for Hg and gene expression, respectively. The Hg in female yellow perch muscle ranged from 0.18 $\mu g \cdot g^{-1}$ to 2.13 $\mu g \cdot g^{-1}$ wet weight (ww) in these lakes. Brain and muscle tissues from twelve other perch collected from four lakes were also analyzed for MeHg and THg. MeHg in perch brain tissues ranged from 0.38 $\mu g \cdot g^{-1}$ to 2.00 $\mu g \cdot g^{-1}$ ww across lakes and significantly correlated with muscle THg and liver MeHg concentrations. The mRNA steady state levels did not correlate with increasing Hg concentration in any tissue; this is consistent with previous findings in which fish showed no changes in gene expression in brain when exposed to MeHg. However, the individual gene expression profiles did correlate with each other. Studies of wild fish have more confounding variables when compared to laboratory exposures, and additional studies should be performed to reduce the effects of these variables and to gain more information on different genes and pathways that may be affected by MeHg toxicity.

The role of introduced smallmouth bass and chain pickerel on mercury transfer in Nova Scotia lakes (PO)

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The lakes of Nova Scotia are often described as being the "perfect storm" for the accumulation of bioavailable mercury due to acidity, dissolved organic carbon and other factors. As a result, many of the fish species can contain higher than normal mercury levels due to the potential for the biomagnification of mercury up through the trophic levels, which would affect the structure of the food web. However, there is still sparse understanding of how non-native piscivorous fish species in Nova Scotia can impact mercury transfer and biomagnification rates in lake food webs. Specifically, smallmouth bass (*Micropterus dolomieu*) and chain pickerel (*Esox niger*) are spreading throughout Nova Scotia and are of major concern in regard to food web and habitat disruption. Blackett Lake (Cape Breton) and Mattatal Lake (Cumberland) are both mesotrophic lakes with residential development and

popular sport fishing destinations. We sampled the fish portion of the food web in both lakes, using stable isotopes of nitrogen and carbon, to compare and contrast the role of smallmouth bass and chain pickerel as indicators of disrupted food web biomagnification. It is expected that mercury would biomagnify throughout the food web in accordance with trophic levels as determined by stable isotope data, and the route of mercury transfer will be impacted by the presence of both non-native piscivorous species.

Bioavailability and nanotoxicity of silver nanoparticles in freshwater mussels (PO)

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The increasing application of silver nanoparticles (nAg) in various consumer products has raised concerns about toxicological impacts in the environment. It is unclear at present whether the toxicity of nAg in mussels is mainly the result of the release of ionic Ag^{1+}. We exposed the freshwater mussel *Elliptio complanata* to increasing concentrations of 20 nm nAg, 80 nm nAg and dissolved Ag^{1+} for 96 hours at 15°C. Following bioavailability assessments, these biomarkers were used to tease out the mode of action of nAg-induced toxic effects: metallothioneins (MT) (ionic Ag^{1+} release), lipid peroxidation (LPO) (ionic Ag^{1+} and nano-surface interactions), heat shock proteins (HSP) (size-related effects), protein-ubiquitin levels (size-related effects) and DNA strand breaks (ionic Ag^{1+} and size effects). Ag was detected in soft tissues with each form of Ag with bioconcentration factors of 20, 9 and 7.2 for Ag^{+}, 20 and 80 nm nAg respectively. Biomarkers revealed that the response pattern of the 80 nm Ag was more closely related to ionic Ag^{1+} than 20 nm Ag^{1+}, suggesting a more important release of dissolved Ag from the 80 nm nAg. The data revealed that all forms of Ag were able to increase the levels of MT and LPO, which suggests that the presence of ionic Ag^{1+} leads to oxidative stress. However, the nanoparticles were able to induce changes in protein-ubiquitin and, to a lesser extent, actinomyosine-ATPase, MT and DNA strand breaks in the digestive gland in a different manner than Ag^{1+}, which permitted the discrimination of the forms of Ag. Moreover, LPO was closely associated with DNA strand breaks in the digestive gland and was not entirely explained by the induction of MT, which suggests another type of toxic interaction. It is concluded that the presence of nAg will not only increase the toxic loadings of released Ag ions, but also generate other and perhaps cumulative effects of nanoparticle-induced toxicity related to the size and surface properties.

Modern Mining

Restoring ecosystem services in mining-impacted regions: The Sudbury case history (PL)

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The "end of the mining cycle" is usually described in terms of meeting government regulations for safe storage of wastes, elimination of surface infrastructure and hazards (open pits, shafts, etc.), and reduction of contaminants to acceptable levels. However, a more modern or perhaps enlightened view of the goals for decommissioning mining sites is the reestablishment of a full suite of ecosystem services: services like provision of clean drinking water, nutrient and carbon sequestration, biodiversity enrichment, agricultural and wildlife production, fish safe for consumption, aesthetics and recreational opportunities. The Sudbury case history provides an opportunity to assess the potential for achieving such valuable goals. The Sudbury site is one of the largest mining complexes on earth with a massive environmental footprint of terrestrial and aquatic damage, the result of over a century of mining and smelting operations. More than $1 billion has been invested in past decades, and similar amounts are committed to continue pollution reduction and land reclamation efforts in the years ahead. Results from intensive monitoring programs that began in the 1970s will be presented to assess the effectiveness of these efforts that appear to be moving progressively from simple "pollution controls" to "reestablishment of valued ecosystem services."

Metal geochemistry of wetlands in watersheds recovering from historical metal and sulphur deposition (PL)

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In watersheds that have been impacted by mining, there is an interaction between biogeochemical cycling, hydrology, and chemistry that influences toxicity of metals for aquatic organisms. Organic soils and wetlands act as natural stores of metal and sulphur and can act as sources or sinks of metals in surface waters, so wetlands play an important role in understanding natural recovery and assisted restoration of mining-impacted areas. Despite this obvious importance, we know little about the functional status and mechanisms controlling the release of metals from wetlands in these disturbed areas. This study investigates metal geochemistry and speciation in six mining-impacted Sudbury peatlands and their associated streams that drain into recovering lakes. The purpose of this study is to determine (1) if the wetlands are losing or retaining metals and (2) what factors control this loss or retention. Spatial and temporal differences in speciation of metals are also explored, with implications to potential toxicity. Annual budgets reveal that these wetlands are releasing metals to

surface waters. These releases are controlled largely by mineralization events and drought-related re-acidification events. These re-acidification events are associated with a high potential for toxicity, as large concentrations of free-ion metals are released. These results offer important considerations for water quality of boreal surface waters in general, but this study also has particularly important implications for restoration efforts in smelter-impacted areas like Sudbury. Efforts to restore aquatic ecosystems in such areas and protect freshwater resources elsewhere must take into account biogeochemical processes within the entire watershed, especially within wetlands.

Does ecosystem disturbance alter the capacity of dissolved organic matter to mitigate the toxicity of copper to *Hyalella azteca*? (PL)

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The potential for aquatic ecosystem recovery as a result of dissolved organic matter (DOM) protecting against metal toxicity has become a significant area of research in environmental toxicology. It is a well-characterized relationship that DOM binds free metal ions, making them unavailable for toxic action such that a reduction in toxicity is seen. Less understood is source variability and how the upland terrestrial environment influences that protective capacity (quality). The aim of this study is to examine DOM quality by comparing the protective capacity of up to 12 natural sources on acute and chronic copper (Cu) toxicity and bioaccumulation in *Hyalella azteca*. Sources included watersheds disturbed by fire, logging, and smelter emissions. Acute (96-hour) and chronic (28-day) toxicity tests were done according to standard (Environment Canada) methods, and were completed in duplicate (acute) or triplicate (chronic) using 10 *Hyalella* aged 2-9 days added to solutions of Cu (0-4 µM) and DOM sources at a dissolved organic carbon (DOC) concentration of 5 mg $C{\cdot}L^{-1}$ (acute) or 7 mg $C{\cdot}L^{-1}$ (chronic). Test solutions were maintained at pH 7.2, 21°C, and 13 $mg{\cdot}L^{-1}$ hardness. Tests showed significant variability among sources, with disturbed sites offering less protection than reference sites. The acute results were complemented with 6-hour Cu uptake/binding experiments and optical characterizations (excitation-emission matrix spectroscopy, absorbance at 340 nm and fluorescent indices). Chronic results were supported by patterns in the dry weight of organisms at day 28. Chronic toxicity was not associated with bioaccumulation of Cu. This project contributes to an improved understanding of DOM quality characteristics, which can potentially be used to improve predictions of Cu toxicity in freshwater ecosystems. Research was supported by NSERC, Vale, and Xstrata within the Terrestrial-Aquatic Linkages for Ecosystem Recovery (TALER) Program.

Effects of the structure of natural organic matter (NOM) on the amelioration of acute nickel toxicity to wild *Daphnia* (PL)

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Natural organic matter (NOM) complexes metals and decreases their toxicity to aquatic biota. While increasing NOM concentration increases mitigation of metal toxicity, NOM structure may also play a role, but this structural effect of NOM on metal toxicity has received little study. Hence, how the structural heterogeneity of NOM affects nickel (Ni) toxicity to a native Canadian *Daphnia pulex/D. pulicaria* hybrid that had been isolated from a lake with a history of metal contamination was investigated. To quantify structure, NOM isolates from thirteen different natural sources were characterized with six different spectroscopic techniques. The addition of 7 $mg{\cdot}L^{-1}$ of NOM decreased toxicity, i.e. increased Ni LC_{50}s; seven of the isolates did so to a significant degree. Both specific absorbance coefficient (SAC) and specific UV absorbance (SUVA) techniques, used as aromatic indices, were positively related to toxicity amelioration. The protective effect of the NOM was best described by segmented regression rather than linear regression, with ameliorative effect of NOM increasing to a plateau at 19.3 $cm^2{\cdot}mg^{-1}$ for SAC and 3.03 $L{\cdot}mg^{-1}{\cdot}m^{-1}$ for SUVA. Phenolic functional groups within NOM structure bind with metals and seem to be the main constituents alleviating metal toxicity. There is growing use of the Biotic Ligand Model (BLM) in setting standards for metal toxicity, and a quality factor (F) has been used to quantify amelioration differences in NOM structure. The addition of the quality factor F improved the relationship between measured and predicted Ni LC_{50}s by 5%.

The role of stream microbial communities in the recovery of aquatic ecosystems from natural and industrial watershed disturbance (PL)

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Microbial communities in streams, by participating in the decomposition and processing of particulate and dissolved organic matter, play a significant role in the recovery of aquatic ecosystems from watershed disturbances. This study investigates the structure and function of microbial communities on a standardized substrate (alder leaves) in streams across a gradient of natural and industrial watershed disturbance. Preliminary results show a range in mean average decomposition rates (3.3-12.8% leaf mass loss), fungal biomass estimates (8.6-164.2 $mg{\cdot}g^{-1}$), bacterial cell counts (5.8-15.7x 105 $bacteria{\cdot}mL^{-1}$), and 16S/18S rRNA pyrosequencing results for the most dominant bacterial and fungal taxa present across undisturbed, logged, fire and industrially disturbed streams. Industrially disturbed streams, in particular, differ in terms of water chemistry (higher levels of heavy metals and urban influences) and microbial community structure (distinct clustering at 20% similarity, low fungal abundance, absence of bacterial genera *Chitinimonas*, a re-calcitrant compound degrader, and dominance of different fungal genera such as the yeast *Cryptococcus*). Lower levels of decomposition and higher levels of microbially derived dissolved organic matter also suggest a difference in microbial

community function in industrial streams. More in-depth analyses of 16S/18S rRNA pyrosequencing data, flourescence analysis, and enzyme assays are ongoing and expected to reveal additional aspects of the functions of the unique microbial communities across disturbance gradients. Understanding the specific microbial contribution to aquatic systems' recovery from watershed disturbance is important in helping to characterize the patterns and processes that promote overall ecosystem integrity and inform restoration strategies. Our inclusion of recovery from natural disturbance (fire in boreal forest watersheds) sets ecologically relevant targets and benchmarks for those restoration strategies.

Spatial and temporal variations in peatland geochemistry in Sudbury, Ontario (PL)

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The damage to the Sudbury landscape from over a century of smelter activity has been severe, and impacts on aquatic and terrestrial ecosystems are well documented. However, despite their abundance in the region, wetlands have received much less attention. The Sudbury Soil Risk Assessment (2001-2008) identified nutrient limitations to be as much of a problem as metal toxicity, and highlighted not only the importance of wetlands but also the need for more detailed studies examining the role of wetlands in the recovery of lakes. The objective of this work is to evaluate the spatial and temporal variability in the geochemistry of 18 wetlands (poor fens) extending along a historic pollution gradient in Sudbury, Ontario. Peat and pore-water chemistry in Sudbury peatlands exhibits large spatial and temporal variability. The historic pollution gradient is still evident in copper (Cu) and nickel (Ni) concentrations in peat, but pore-water chemistry is also strongly influenced by natural factors such as groundwater and peat carbon content. Redox processes contribute greatly to temporal variations in pore-water chemistry. The August and October campaigns were characterized by higher SO_4, lower pH and higher concentrations of metals such as Ni and cobalt (Co) compared with the May campaign. Despite the large spatial and temporal variability in pore-water chemistry, soil-solution partitioning of some metals can be explained by pH alone. Modeling is significantly improved with the addition of predictors representing dissolved organic matter (DOM) quality and quantity, SO_4 and ä18O values. Gaining a thorough understanding of wetland geochemistry can provide valuable insights into a wetland's contribution to and influence on ecosystem health and can allow for effective site-specific and seasonal-specific remedial measures in wetlands impacted by over a century of mining activities.

Assessing the effects of total dissolved solids from the Snap Lake diamond mine on freshwater fish and their prey (PL)

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As a result of deep, relatively saline connate water that is released with treated mine water into Snap Lake, the total dissolved solids (TDS) concentration of the lake water is increasing. Toxicity of TDS to aquatic biota is dependent on its specific ionic composition. Thus, although available literature indicated that resident biota should not be adversely affected by TDS concentrations of 500 $mg{\cdot}L^{-1}$ or greater, testing with representative fish and their prey was required to determine an appropriately protective site-specific water quality objective for the TDS in the lake water. Laboratory toxicity tests were conducted within the most sensitive life stages of two fish species found in the lake (Arctic grayling, *Thymallus arcticus*, and lake trout, *Salvelinus namaycush*): egg fertilization, hatching, and fry development. Laboratory toxicity tests measuring acute and chronic endpoints were also conducted with key components of the lake food chain (algae, plankton, benthos). All biota tested had tolerances above 500 $mg{\cdot}L^{-1}$ and, except for two waterflea species, also had tolerances above 1000 $mg{\cdot}L^{-1}$.

The importance of considering baseline conditions and environmental assessment predictions in Environmental Effects Monitoring programs associated with the *Metal Mining Effluent Regulations* (PL)

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The current *Metal Mining Effluent Regulations* (MMER) Environmental Effects Monitoring (EEM) program determines effects on fish populations and fish habitat based on statistical comparisons of reference and exposure areas. Environment Canada's EEM guidance document suggests that baseline conditions can be considered when conducting a study, but the language in the regulations specifically excludes consideration of this essential information. In addition, the MMER and the EEM guidance document do not consider the effect predictions made in government-approved environmental assessments. Unlike the EEM program, environmental assessments are risk-based evaluations. All EEM-defined effects are treated equally when they should not be. Discussion will focus on real-world examples, implications of the current regulations, and recommendations to enhance this vital monitoring program.

Do the water chemistry parameters pH, alkalinity, and dissolved organic carbon affect the bioaccumulation and toxicity of metals in a metals-contaminated system? A case study (PL)

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A northern Quebec lake system impacted by acid mine tailings for 70 years was sampled in 2011 and 2012. Alkalinity, dissolved organic carbon (DOC), and pH were measured. As well, major ions and up to 45 dissolved metals were measured across the system. In conjunction, *Hyalella azteca* sediment toxicity tests, waterborne toxicity tests, and some *in situ* cage tests were performed to assess toxicity and metal bioaccumulation. The data will be presented and the relationships of pH, alkalinity, and dissolved organic carbon to the bioaccumulation and toxicity of metals in the amphipods will be examined. Results obtained from laboratory testing of the effect of alkalinity, DOC, and pH on the bioaccumulation and toxicity of cobalt (Co) will be compared to field results to determine if laboratory-based models can be used to predict field effects.

A novel laboratory-based approach for establishing site-specific water quality guidelines for selenium (PL)

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Water quality guidelines for selenium (Se) are most appropriately established on the basis of concentrations of selenium in tissues of aquatic organisms, such as fish. However, there is often a need to establish water column concentrations of selenium that correspond to tissue limits, so that risk associated with present or future activities can be appropriately evaluated. Establishing appropriate water column limits for selenium is complicated by significant site-to-site variability in uptake of selenium into primary producers and higher trophic levels, which obfuscates predictions of the relationship between water column and tissue concentrations of selenium at a particular site. A laboratory-based method to measure site-specific bioconcentration rates of selenium into primary producers is presented here which can be used to measure site-specific differences in accumulation rates of selenium. Case studies are presented that use this information to calculate site-specific water quality benchmarks for selenium.

Dietary selenomethionine exposure alters swimming performance, metabolic capacity, and energy homeostasis in juvenile fathead minnow (PL)

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Selenium (Se) is known to cause chronic toxicity in aquatic species. In particular, dietary exposure of fish to selenomethionine (SeMet), the primary form of Se in the diet, is of concern. Recent studies suggest that chronic dietary exposure to SeMet alters energy and endocrine homeostasis in adult fish. However, little is known about the direct effects of dietary SeMet exposure in juvenile fish. The objective of the present study was to investigate sublethal physiological effects of dietary SeMet exposure in juvenile fathead minnow (*Pimephales promelas*). Twenty days-post-hatch fathead minnow were exposed for 60 days to different measured concentrations (2.8, 5.4, 9.9, 26.5 μg $Se{\cdot}g^{-1}$ dry weight) of Se in food in the form of SeMet. After exposure, samples were collected for trace metal analysis and fish were subjected to a swimming performance challenge (critical swimming speed, U_{crit}). A decrease in critical swimming speed (U_{crit}) occurred in the 9.9 and 26.5 μg $Se{\cdot}g^{-1}$ exposure groups compared to the control group. Tail-beat amplitude and frequency were decreased in both the 9.9 and 26.5 μg $Se{\cdot}g^{-1}$ exposure groups compared to the control group. An increase in oxygen consumption (MO2) and cost of transport (COT) was observed in the 9.9 and 26.5 μg $Se{\cdot}g^{-1}$ exposure groups compared to the control group. The active metabolic rate of the high dose group was increased relative to controls, but this did not translate into a significant increase in the aerobic scope. Energy storage capacity was measured via whole-body glycogen and triglyceride concentrations. Triglyceride concentrations in non-fatigued fish were elevated in the 5.4 μg $Se{\cdot}g^{-1}$ group relative to controls. Glycogen concentrations in the 5.4 μg $Se{\cdot}g^{-1}$ group were increased in the fatigued fish versus the non-fatigued fish. The stress biomarker cortisol will also be determined in fatigued and non-fatigued fish. The results from this study will be used to gain new insights into the sublethal effects of dietary Se exposure on a juvenile fish species.

Quantifying the importance of physicochemical sediment characteristics in altering uranium bioavailability in reconstituted *vs.* natural freshwater sediments (PL)

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Sediments are a reservoir for most metals; however, our knowledge and understanding of the role of sediment physicochemical characteristics in altering the bioavailability and toxicity of some metals is incomplete. This is particularly true for uranium (U) which, when discharged to aquatic systems, is often associated with sediment due to its high sediment partition coefficient. The goal of this research is to quantify the role of different sediment characteristics in the bioavailability, and hence toxicity, of sediment-associated U to a model freshwater benthic invertebrate. To achieve this, freshwater midge (*Chironomus dilutus*) larvae were exposed in several 10-day tests to both reconstituted and field-collected reference sediment spiked with U to describe differences in U

bioaccumulation from sediments with different physicochemical properties, specifically particle size distribution and total organic carbon (TOC) content. Reconstituted sediments were prepared to mimic the physical and chemical properties of field-collected sediments in order to quantify the influence of different sediment characteristics on U bioavailability with fewer artifacts than would be associated with natural sediment. Tests were conducted with sublethal U concentrations of 5 to 200 mg $U{\cdot}kg^{-1}$ dry weight (dw) in sediments aged for 20 days. Test endpoints and measurements included midge survival and growth, and U concentrations in whole organisms, whole-sediment, overlying water and pore water. In all tests, *C. dilutus* survival was above 80% regardless of sediment type, U concentration, or physicochemical characteristic. Results indicate that increasing quantities of either clay or TOC content can significantly reduce U bioavailability in both reconstituted and natural sediment. An increase from <1 to 18% TOC in sediment spiked with 50 mg $U{\cdot}kg^{-1}$ dw resulted in a reduction in U bioaccumulation from 69.0±4.1 to 9.2±2.4 mg $U{\cdot}kg^{-1}$ dw and from 74.1±10.1 to 10.8±7.0 mg $U{\cdot}kg^{-1}$ dw in midge exposed to natural and reconstituted sediment, respectively. Similar results were observed for clay content, suggesting that both particle size distribution and TOC content play important roles in modifying U bioavailability and should be considered when evaluating the risk of U in aquatic environments.

Selenium in otoliths of creek chub and green sunfish from a coal mining-impacted reach of the Mud River, West Virginia (PL)

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Establishing the exposure histories of wild fish to trace elements is often difficult for mobile species. Chemical analyses of muscle or visceral tissues can provide information on recent exposure, but depuration, metabolic transformation, and tissue redistribution preclude temporal resolution. Otoliths, the calcified structures in the inner ear of teleost fish, are considered to be metabolically stable and therefore may serve as continuous records of exposure to trace elements in the environment. Otoliths are composed of layers of aragonite in a protein matrix deposited annually throughout the lifetime of the fish, thus providing a time scale. Both the inorganic portion and protein matrix have the capacity to incorporate a wide range of trace elements and, as such, otoliths may be used to determine a fish's history of contaminant exposure in the wild. The Mud River ecosystem in West Virginia has been impacted by selenium (Se) emanating from mountain top removal/valley fill coal mining. Otoliths from creek chub (*Semotilus atromaculatus*) and green sunfish (*Lempomis cyanellus*) were analyzed using laser ablation inductively coupled mass spectrometry (LA-ICP-MS) to determine Se concentrations. As part of a larger study examining trophic transfer and bioaccumulation of Se, each fish's capture location and the concentration of Se in its otoliths is described in relation to adjacent coal mining activity. This study represents the first determinations of Se in otoliths of creek chub and green sunfish. Preliminary results indicate that Se is present in otoliths of fish captured from mining-impacted sites to a greater extent than in fish from reference sites.

Effect of total dissolved solids on fertilization and development of two salmonids (PO)

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Salmonids have been reported to be sensitive to effects associated with total dissolved solids (TDS) in some previous tests, with fertilization reported to be a particularly sensitive life stage in some cases. There is general agreement that, of all fish life stages, early life stages are the most sensitive to TDS. The testing reported here evaluated the effect of TDS related to the Snap Lake Diamond Mine (Northwest Territories, Canada) on lake trout (*Salvelinus namaycush*) and Arctic grayling (*Thymallus arcticus*) in exposures encompassing the embryo-alevin-fry early life stages. Two exposures were conducted with each species: one was initiated prior to fertilization, and the other subsequent to fertilization. For these species, fertilization was not adversely affected by TDS related to the mine at concentrations >1400 $mg \cdot L^{-1}$. For the specific TDS composition tested, these two fish species were less sensitive than water fleas and, at the highest tested concentrations, showed a lack of effects, as did a diatom, an alga, a rotifer and a chironomid.

Using molecular biomarkers and traditional morphometric measurements to assess the health of slimy sculpin from streams with elevated selenium in north-eastern British Columbia (PO)

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Fish-based Environmental Effects Monitoring (EEM) programs use individual- and population-level endpoints to assess aquatic health. The impacts of coal mining and elevated levels of selenium (Se) were assessed in slimy sculpin (*Cottus cognatus*) from two reference streams (inside and outside of coal zone) and one stream with a history of coal mining, using traditional EEM endpoints (condition factor, gonadal somatic index and liver somatic index). To determine if the assessment of impacts could be improved by including molecular biomarkers, real-time PCR assays were optimized for genes associated with reproductive endpoints (vitellogenin, estrogen receptor α, steroidogenic acute regulatory protein, aromatase, and glycogen synthase). Oxidative and cellular stress endpoints (superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, and heat shock protein 90) were also measured, as Se exposure can cause oxidative stress. Water Se levels exceeded guidelines in the stream with historical mining (4 $\mu g \cdot L^{-1}$), but benthic macro-invertebrates did not exceed dietary thresholds (2-3 $\mu g \cdot g^{-1}$ dry weight [dw]). Whole-body Se levels were above British Columbia's guideline in slimy sculpin from all streams, but only above the draft U.S. Environmental Protection Agency criterion (7.91 $\mu g \cdot g^{-1}$ dw) in fish from the reference stream inside the coal zone. Some transcripts (sod, gpx) involved in cellular and oxidative stress were elevated in slimy sculpin collected at the impacted site, suggesting that Se exposure may be increasing reactive oxygen species production; however, traditional and

molecular differences in growth, reproduction, and condition biomarkers appeared to be driven by food availability, and not Se exposure.

The role of Ca^{2+}, Mg^{2+} and dissolved organic matter as toxicity-modifying factors in boreal shield lakes (PO)

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This study is focused on the influence of Ca^{2+} and natural organic matter (NOM) on the acute and chronic toxicity of nickel (Ni) to *Hyalella azteca*. Amphipods (source: Hannah Lake, Sudbury) were cultured and tested in soft water (0.1 mM Ca, 0.025 mM Mg, 0.1 mM Na, 0.1 mM Cl, pH 7, temperature 20°C) following Environment Canada standard methods. In acute exposures, increasing Ca (0.1 - 2.0 mM) significantly reduced Ni toxicity by six-fold (LC_{50} values increased from 0.56 $mg \cdot L^{-1}$ to 3.2 $mg \cdot L^{-1}$). NOM at concentrations above 6 mg $C \cdot L^{-1}$ also showed significant protective effects. NOM collected from various sites across Ontario represented smelter impacted lakes (Laurentian Lake, a wetland from Laurentian Lake, Daisy Lake), fire-impacted lakes (White River site 2), logging-impacted lakes (White River site 3), and reference lakes (White River site 1, Harp Lake, a stream flowing into Harp Lake, Clearwater Lake, Plastic Lake, a stream flowing out from the wetland, and a stream flowing into the wetland). Tests with collected NOM showed that some sources offered more protection than others. Short-term accumulation was used to establish linkages between toxicity mitigation and bioavailability/uptake. Targeted chronic testing was done to establish whether protective effects are comparable to acute exposures. Chronic effects of Ni were assessed through growth and survival of organisms. These results are considered in the context of the biotic ligand model and understanding the importance of NOM source. This study was funded as a Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development project, with contributions from Vale, Xstrata, and the City of Sudbury.

Freshwaters

Using remotely-sensed data to assess changes in the Petitcodiac River sediment plume in the upper Bay of Fundy, Canada, prior to and following a causeway removal project (PL)

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The Petitcodiac River Causeway Project is a multi-stage, large-scale environmental restoration project being carried out on the tidally influenced Petitcodiac River in New Brunswick, Canada. The Petitcodiac River Causeway is a causeway and concrete dam with flood control gates that was constructed in 1968 on the Petitcodiac River. Since the completion of the causeway in 1969, fish migration has been impeded and does not meet the fish passage requirements of the *Fisheries Act* administered by Fisheries and Oceans Canada. An Environmental Impact Assessment determined that the causeway should be removed and a bridge installed, requiring a monitoring program to establish baseline conditions for comparison against post-gate opening physical conditions within the receiving environment of the upper Bay of Fundy. During pre-gate opening conditions, the surface sediment plume from the Petitcodiac River could be observed in satellite images at the point where the river met the coast; the plume extended into the upper Bay of Fundy, in close proximity to commercial fishing grounds. Concern focused on the potential for sediment loading to increase on commercial fishing grounds with the causeway removal. Using remotely-sensed data and GIS mapping, the maximum extent of the sediment plume in the receiving waters of the upper Bay of Fundy was estimated prior to and following the opening of the causeway gates. Specifically, remote sensing techniques were employed to measure and map total suspended solids (TSS). Qualitative and quantitative methods were developed using the satellite reflectance response data from Landsat and SPOT satellites, which span a 27-year period (1984 to 2012). A time series was developed that delineated the sediment plume in the receiving waters of the upper Bay of Fundy, starting with causeway gate-closed conditions and extending to gate-open conditions. This advancement in the application of remotely sensed data to a highly turbid coastal environment allowed the monitoring of far-field effects of a large-scale environmental restoration project. The information gathered from this project will aid in making informed decisions and has helped in obtaining historical data about TSS in the project area, which were not previously available.

Fate and prevalence of antibiotic resistance genes in mescocosm and constructed wetland systems in a Canadian Prairie context (PL)

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The global emergence of antibiotic-resistant microbes requires mitigation strategies to minimize their release and potential impacts on surface-water quality and their downstream implications for human health and the environment. Antibiotic resistance genes (ARGs) in bacterial hosts can enter natural waters via wastewater. The discharge of complex mixtures of nutrients, organic micropollutants, and ARGs from municipal treatment lagoons into lotic systems remains a concern for human and aquatic health on the Canadian Prairies. The rural community of Grand Marais, Manitoba, passively treats wastewater in sewage lagoons and a treatment wetland before discharging into surface waters that flow directly into Lake Winnipeg. Using this facility as a model system, the study assessed the presence of pharmaceuticals and ARGs in lagoon outputs and potential removal by the wetland. A parallel mesocosm study using simulated wastewater similarly evaluated the fate of pharmaceuticals and ARGs in wetland systems planted with emergent (*Typha* spp.) and submergent (*Myriophyllum sibricum*) macrophytes compared to unplanted controls. Quantitative PCR measured the abundances of ten ARGs, as well as 16S-rRNA, to evaluate the effectiveness of the wetlands in removing ARGs. Pharmaceuticals were detected at concentrations in the $ng{\cdot}L^{-1}$ range in effluent from Grand Marais, with some attenuation observed as the effluent moved through the wetland system. The system, however, did not significantly remove ARGs in the wetland, and up to 2.7% of the bacterial population carried ARGs. No correlation existed between concentrations of sulfonamide drugs and prevalence of sulfonamide resistance genes. In the mesocosm study, the tanks receiving wastewater consistently had elevated abundances of ARGs compared to controls. Similar to the wetland, the presence of plants or addition of pharmaceuticals did not affect the rate of removal of ARGs from the system. The results of these studies indicate that surface-water treatment wetlands do not specifically target ARGs for removal. Additional studies would be beneficial to determine whether upgrades to extend retention time or alter plant community structure within a treatment wetland would optimize removal of ARGs.

Aquatic community metabolism applicability for use as a monitoring tool in Canadian rivers (PL)

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Traditional river health indicators are based on structural measurements, such as species diversity and abundance, and biophysical parameters, such as dissolved oxygen concentrations. However, functional measurements, such as rates, patterns, and ecological processes, are also required for a more complete assessment of river health. River metabolism is a desirable measure of river health because it measures the rates of production/respiration and use of organic carbon, thereby providing a

direct estimate of food web function. It is affected by both natural and anthropogenic factors, and can reflect system-level responses to external stressors. Aquatic metabolism studies have been conducted for 80 years and, during this period, the technology has improved from oxygen measurements using light/dark incubation of bottled water to continuous real-time, *in situ* diel oxygen measurements of waterbodies. The state of river metabolism knowledge, coupled with advances in technology, is now at a point where we can use metabolism as an indicator of the health status of a river and provide diagnostic information as to the cause of stress in the system. The focus of our research has been to determine whether community metabolism using the diel oxygen method can be accurately determined in Canadian rivers and streams, and whether it is feasible to use metabolism as a diagnostic bioassessment tool. We undertook community metabolism studies on rivers and streams of the St. John River, Lake Erie, and Lake Winnipeg watersheds. This talk will discuss these projects, their successes and challenges and future recommendations.

Chemodynamic behavior of thallium in the Slave River, Northwest Territories, Canada (PL)

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The environmental fate and effects of thallium (Tl) are probably the least understood of any of the contaminant heavy metals. Major anthropogenic sources of thallium in the environment include fossil fuel extraction and combustion and potassium-based fertilizers. The Slave River in Canada's Northwest Territories drains large portions of northern Alberta and British Columbia via its two major tributaries, the Peace and Athabasca rivers. In the course of studies of the health and contaminant status of fish in the Slave River, we have observed consistent increases in the tissue concentrations of thallium in some fish species in the lower Slave River compared to tissue concentrations in the Peace and Athabasca rivers. These increases seem to be most marked in higher trophic level species, e.g. northern pike (*Esox lucius*) and walleye (*Sander vitreus*), but are also apparent in lower trophic level fishes. With the presumed source of the Tl in the Slave system being hydrocarbon extraction operations in Alberta, it is unclear why increases in fish tissue concentrations are seen mainly in the lower Slave River. It is possible that alterations in the oxidation state or other speciation phenomena occur in the upper Slave River, resulting in differential accumulation efficiencies between the river's upper and lower stretches. The higher concentrations observed in higher trophic level species suggest the potential for biomagnification of an organic form of the metal. While the concentrations of Tl in fish tissues do not represent an immediate health concern to consumers, this phenomenon offers an opportunity to further investigate the environmental chemodynamics of this poorly understood element.

A community-based cumulative effects monitoring program for the Slave River, Northwest Territories, Canada (PL)

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The Slave River Delta is one of Canada's largest freshwater delta systems. The Slave River is fed by the Athabasca and Peace rivers, which flow through northern Alberta. Increased industrial activity in the Peace/Athabasca river basin has been a source of ongoing concern to First Nation and Métis communities for whom the Slave River and Delta are part of a traditional way of life. Concerns about water quality and its potential human and wildlife health effects have been frequently expressed by these communities and have been instrumental in the development of the Slave River and Delta Partnership (SRDP), an ongoing partnership of communities, First Nations and Métis leadership, territorial and federal agencies, non-governmental organizations (NGOs), and "western scientists." In addition to industrial activities on water quality and the hydrodynamics of the river/delta, there are concerns about the cumulative and interactive impacts of these and other stressors, particularly global climate change. To address the concerns of the communities, we have developed, in partnership with the SRDP, a community-based monitoring program working with communities, local territorial and federal agencies, NGOs and researchers. The Slave Watershed Environmental Effects Program (SWEEP) is developing a series of indicators that will integrate local and traditional knowledge and western science measures. The findings will be integrated using a Bayesian Belief Network (BNN) and will be freely available to communities and regulators, with emphasis put on timeliness of delivery and presentation of the results in an easily understood manner. Indicators are being developed to address water quality, hydrodynamics and ice dynamics, wildlife impacts, fish health and quality, and human dimensions of the changes being observed. It is hoped that the types of bioindicators being used and the means of integration in the BNN for community presentation will provide an example for the development of other community-based monitoring programs.

Green de-icing compounds: Are they really green? (PL)

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Road salt (sodium chloride, NaCl) used for deicing in winter is recognized as a major contributor to increasing salinization of surface and ground waters in northern latitudes. The low cost, availability, and high usage of NaCl on roads has resulted in rates of salinization that threaten availability of fresh water in Eastern Canada and the northeastern United States. Aquatic organisms inhabiting roadside wetlands are particularly susceptible to the toxic effects of chloride in runoff water. Soils, roadside vegetation, and infrastructure are also negatively affected by road salt. A number of "greener" alternatives to NaCl are in current use as deicing compounds, including acetates, formates, urea, and

other Cl- compounds. Calcium magnesium acetate (CMA), in particular, is proposed as a greener alternative to NaCl due to its low toxicity in water and its rapid degradation. A series of acute toxicity tests (LC_{50}) were performed with seven current-use deicers on larvae of a number of native amphibian species. Results indicate differential toxicities of the various deicers to the amphibian larvae, with American toads being generally the most tolerant. While there are apparent differences in toxicities of Cl- based deicers in the laboratory, actual toxicities of these compounds in the environment are closely related to appropriate application on roads. A number of "green" deicers function as nutrients in freshwater systems, increasing biochemical oxygen demand (BOD) and potentially leading to hypoxia in the small wetlands inhabited by many amphibians.

Long-term effects of wildfire on sediment-associated metal toxicity and bioaccumulation in the Crowsnest River, Alberta (PL)

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There is increasing global recognition of the effects of large-scale land disturbance by wildfire on a wide range of water and ecosystem services. In 2003, the Lost Creek wildfire burned a contiguous area of 21,000 ha on the eastern slopes of the Rocky Mountains in southern Alberta. This disturbance significantly impacted the water quantity and quality of downstream river reaches and reservoirs. Previous research reported that metal levels in rivers draining burned landscapes were 2-15 times greater than in unburned reference streams (Silins et al. 2009). Currently there is no information on the long-term effects of wildfire on the bioaccumulation and toxicity of sediment-associated metal in fire-impacted streams in Alberta. The toxicity and bioaccumulation of particulate-associated metals in wildfire-impacted streams in southern Alberta were evaluated in this study. Phillips samplers were deployed to collect particulates in streams draining burned and reference (unburned) zones within the area impacted by the Lost Creek wildfire. Toxicity and bioaccumulation were determined in the laboratory using the epi-benthic freshwater amphipod, *Hyalella azteca*. A metal effects addition model (MEAM) was used to assess the impact of metal mixtures and to predict chronic mortality (Norwood et al. 2013). Increased concentrations of barium (Ba), chromium (Cr), nickel (Ni), lead (Pb), and zinc (Zn) were found in the tissues of *H. azteca* exposed to particulates from burned watersheds in comparison to the unburned watersheds. *H. azteca* survival was similar in exposures to both the burned and unburned sites, indicating that there was little impact due to the burning. However, one burned site (B1), where the observed survival was reduced, was inconsistent with survival predicted by MEAM. The data suggest that factors other than the metals examined were impacting the survival of *H. azteca*. While the levels of sediment-associated metals have decreased in the nine years since the wildfire, some metal toxicity was still observed in *H. azteca*.

Food quantity affects the sensitivity of daphniids in Muskoka Lakes to road salt (PL)

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This study examines the effects of food quantity on the chronic (14-day) toxicity of road salt to daphniids in soft-water lakes. Chronic bioassays were conducted that exposed a *Daphnia pulex/D. pulicaria* hybrid (McFarlane) clone to various combinations of food and chloride salts ($CaCl_2$ and NaCl) in FLAMES media, a bioassay and culture medium designed to mimic the soft-water chemistry of lakes in Muskoka, Ontario. The intrinsic rate of growth (r) and chronic LC_{50} for chloride were determined for each treatment combination, and the number of eggs and neonates was recorded over 14 days. Food quantity was measured as the amount of carbon (as particulate carbon, i.e. algae) per litre. There was a strong linear relationship ($R^2 = 0.9323$ for $CaCl_2$ and $R^2 = 0.9118$ for NaCl) between food quantity and the chronic LC_{50} for chloride. As food quantity increased, the toxicity of chloride to daphniids decreased. The greatest effect of food and chloride on the reproduction of daphniids was on neonate release. Egg production was often not accompanied by neonates release, even though the lipid drop size in eggs suggested they were well nourished. The difference between egg production and neonate release in daphniids exposed to chloride was amplified as food levels decreased. Our research suggests that the nutrient status of soft-water lakes should be considered in the assessment of the threat of road salt pollution to freshwater aquatic organisms.

Different responses in toxicity testing among two members of the *Hyalella azteca* species complex (PL)

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Hyalella azteca (Saussure, 1858), an amphipod crustacean, is frequently used in freshwater toxicity tests involving metals, pH, organic contaminants and sediments. Numerous organizations have collected and established cultures of *H. azteca* originating from localities across North America. However, *H. azteca* is actually a cryptic species complex whose members satisfy both the biological and the phylogenetic species concepts. As a result of morphological similarities among these lineages, they have been misclassified as a single species. The variability among toxicity assays from laboratories across North America may be due to the potential use of different species from this complex. In the present study, two cryptic species, clades 1 and 8, are delineated using DNA barcoding. These lineages of *H. azteca* are exposed to the same 14-day single metal toxicity tests using copper (Cu) or nickel (Ni). The rates of mortality, inhibition of growth, as well as metal bioaccumulation are used as a measure of sensitivity. Concerning these endpoints, with few exceptions, the two species of *H. azteca* have statistically different responses. As a result, genetically characterized cultures of *H. azteca* should be used in toxicity investigations.

Changes in environmental attitudes of industry: Past motivation and future direction (PO)

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In the early days of North American settlement, industry was encouraged by government to utilize natural resources and bring development and wealth to communities. As populations grew and other demands developed, regulations were created to protect natural resources for multiple uses. Industry then argued that environmental regulations increased costs and reduced their ability to compete nationally and internationally. It is only over the last 50 years that environmental regulations and legal precedents have placed substantial responsibility for the protection of natural resources on industry. In recent decades, product advertising and marketing has engaged the consumer as another stakeholder in shaping industry's attitude regarding environmental management and protection. The financial model for business has expanded with a growing economy and an increase in stakeholders. Fuller cost accounting, incorporating the expense and benefit of environmental protection and remediation, has changed the equation of profitability. The corporate need to make a profit for investors now includes avoidance of environmental infractions and subsequent penalties, including fines, loss of market share, decrease of share value/market capitalization and resulting potential acquisition. Corporations now neglect environmental protection at their peril.

Salty Waters

Blue mussels (*Mytilus edulis*) as bioindicators of stable water quality in Sydney Harbour during remediation of the Sydney Tar Ponds, Nova Scotia, Canada (PL)

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Abstract

Contaminants were measured in blue mussels (*Mytilus edulis*) in Sydney Harbour during remediation of the Sydney Tar Ponds (STP) to assess the spatio-temporal distribution of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and metals during baseline and remediation. Overall distribution of chemicals in mussels was compared to contaminants in other marine indicators. Metal concentrations in mussels showed some minor temporal variability, but did not appear to be directly related to remediation activities. Contaminants showed stable or decreasing concentrations, except lead (Pb) and zinc (Zn). Individual PAH compounds were mostly undetected, except for fluoranthene and pyrene. Concentrations of fluoranthene in mussels and water were weakly related ($R^2 = 0.72$). PCBs were undetected, except during year 2 remediation at some near-field stations. Contaminants measured during this study were much lower than previously reported in other studies of mussels in Sydney Harbour, likely due to ongoing natural recovery and because of environmental mitigation measures implemented during remediation activities at STP. The lack of detection of most individual PAHs and PCBs, and low bioaccumulation of metals during baseline and remediation using mussels as bioindicators, reveal subtle improvements in environmental quality in Sydney Harbour.

Introduction

Sydney Harbour, Nova Scotia, has been subject to effluent and atmospheric inputs of chemical contaminants including metals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) from a large coking and steel manufacturing facility that operated in Sydney from 1901 until closure in 1988. Contaminants were discharged into the Sydney Tar Ponds (STP) within a tidal tributary, Muggah Creek, located in Sydney Harbour. Thousands of tons of metals, PCB- and PAH-contaminated sludge were released into Muggah Creek and Sydney Harbour during the plant operation (Furinsky 2002). Many of these particle reactive contaminants were bound to fine-grained, organic-rich particles suspended in the water column and were deposited in harbour sediments, creating the main pathway of exposure, particularly for marine organisms (Stewart et al. 2002; Tay et al. 2003).

Mussels are well-known bioindicators of water quality (Angelo et al. 2007), as their tissues provide information on bioavailability of chemicals in the water column and are well suited to measuring quality of coastal environmental effects (Chase et al. 2001). Because mussels are sessile filter-feeders with a limited ability to metabolize contaminants, they tend to accumulate contaminants to levels exceeding those found in ambient seawater, where concentrations of many contaminants are often

below instrument detection limits (DL). The use of mussels for *in situ* exposures has been successful in evaluating potential long-term effects associated with changes or improvements in environmental conditions in the Bay of Fundy and in the Gulf of Maine Mussel Watch program (Chase et al. 2001). Monitoring programs are commonly used when long-term evaluations of continuous inputs of known stressors are required, such as those from point source discharges when establishing baseline conditions or when evaluating conditions before, during, and after remedial actions.

The governments of Canada and Nova Scotia committed to remediating the STP site in order to reduce potential ecological and human health risks to the environment. An Environmental Impact Statement (EIS) concluded that remediation would likely not cause significant negative environmental impacts with implementation of appropriate environmental management and mitigation protection (AMEC 2005). A follow-up Environmental Effects Monitoring (EEM) program was established to determine the effectiveness of measures to mitigate potential adverse environmental effects of remediation and to verify EIS environmental effects predictions. This study consisted of monitoring contaminants within blue mussels (*Mytilus edulis*) during remediation of one of Canada's most contaminated sites, and comparing with baseline data. Mussels' exposure to marine receiving water in Sydney Harbour was assessed by measuring concentration of metals, PCBs, and PAHs in their tissue. Mussels were suspended for up to nine months at stations in Sydney Harbour distributed at increasing distances from Muggah Creek. Remediation began in 2009 and the main objectives of this study were to: (i) monitor potential effects of remediation on the marine environment using blue mussels (*M. edulis*) as bioindicators; and (ii) compare conditions documented in previous studies to confirm temporal stability in Sydney Harbour.

Methods

Marine monitoring stations (~10 m deep) were established and sampled by boat during one baseline year and three years of remediation between September 2008 and August 2012. Stations were distributed across four assessment areas to reflect the harbour ecosystem, as follows: area 1 – near-field (four stations), area 2 – mid-field (four stations), area 3 – far-field (two stations), and area 4 – Sydney River Estuary (one station). These are described in greater detail in Walker et al. (2013a, 2013b). Commercially available adult blue mussels (50–65 mm shell length) were used. Fisheries and Oceans Canada (DFO) scientific licences were obtained prior to transport and deployment of mussels. Approximately 200 mussels were deployed at each station, attached to frames suspended ~7 m below water surface. Details of mussel and frame deployment can be found in Walker and MacAskill (2013). Two mussel sample collections were conducted following a 60-day and a 9-month over-winter deployment. Approximately 40 mussels were removed from frames in the fall after 60 days (representing the end of the growing season) following the Mussel Watch and Gulf of Maine Council (GOMC) standard methods (Angelo et al. 2007; GOMC 2013). A sub-sample of pre-deployment mussels was analyzed for identical chemical parameters to assess pre-exposure concentrations in mussel tissue. Detailed methods of tissue and water quality analysis can be found in Walker et al. (2013c). Minitab® statistical software was used to determine significant differences both spatially (between stations) and temporally (between years) by one-way ANOVA followed by Tukey's test at the $p < 0.05$ level ($n = 6\text{-}11$).

Results and Discussion

Biofouling from invasive species of tunicates (*Ciona intestinalis*) may have contributed to mussel mortality during the 60-day and over-wintering periods (~20%) (Clancey and Hinton 2003). There were no significant differences in mussel survival between stations or assessment areas or between years ($p > 0.05$). Other potential causes of mortality (not necessarily attributable to water quality) may include crab predation, disease, and physical environmental conditions such as sewage organic inputs to the harbour (Stewart et al. 2002). Table 1 shows chemical concentrations in pre- and post-deployment mussels and compares them to Canadian guidelines for chemical contaminants and toxins in fish and fish products (Canadian Food Inspection Agency [CFIA] 2011) and other studies. Generally, individual PAH compounds were mostly undetected (< 0.05 $\mu g \cdot g^{-1}$), except for fluoranthene and pyrene, plus some minor detections of six other individual compounds. During baseline and remediation years 1 and 2, there were some minor detections of fluoranthene and pyrene at near-field stations. Fluoranthene concentrations were moderately related in mussels and deep water ($R^2 = 0.72$), but not in shallow water ($R^2 = 0.08$) (Figure 1). Pyrene was unrelated in both deep and shallow water samples ($R^2 = 0.35$ and 0.21, respectively, data not shown). Fluoranthene and pyrene concentrations in mussels showed marked spatial variation at stations 4-1 and 1-2, which were almost a magnitude higher than at reference stations. Ernst et al. (1999) measured PAHs in wild mussels in Sydney Harbour during 1995 (0.78 – 5.00 $\mu g \cdot g^{-1}$), which were up to 100 times higher than current PAH concentrations. These results provide evidence of improvements to water quality since cessation of coking and steel mill activities in 1988.

PCBs were not detected (< 0.05 $\mu g \cdot g^{-1}$) in mussels during baseline or years 1 and 3 remediation, but were detected during year 2 remediation at near-field stations, albeit at very low concentrations (0.05-0.07 $\mu g \cdot g^{-1}$). PCB concentrations were well below Canadian guidelines (2 $\mu g \cdot g^{-1}$) for chemical contaminants and toxins in fish and fish products (CFIA 2011; Table 1). A similar spatial pattern was also observed with PCB concentrations in rock crabs in Sydney Harbour during the same period (Walker et al. 2013d), although PCBs were not observed in water samples (Dillon 2013).

Metal concentrations in mussels showed little spatial variation across stations, and more specifically, with distance from the STP site (Figure 2). This lack of spatial variation suggests that the STP site is not the primary source for metals in mussel tissue during remedial activities. In fact, some of the highest metal concentrations measured in mussels were found at reference stations or station 4-1 near the Sydney River. All parameters measured in mussels at near-field stations were below CFIA guidelines, and often contaminants in mussels were lower than pre-deployment concentrations, particularly Pb, meaning that mussels suspended in Sydney Harbour met federal guidelines suitable for human consumption. This is a marked improvement on historical conditions; however, further study is required to assess the chemical contaminant burden on wild mussels in Sydney Harbour, which were not measured as part of this study. Some of the highest metal concentrations were measured at reference stations, and the pattern of elevated concentrations observed in studies of contaminants in sediment, usually centred on Muggah Creek (Smith et al. 2009; Walker et al. 2013a), was not apparent for metal concentrations in mussels in this study. Measured metal concentrations in mussel tissue, albeit low, showed some temporal variability (four years), but these did not appear to be directly related to remediation activities. Concentrations of PAH, PCB, and metals reported here in Sydney Harbour were

compared to other studies of mussels (Table 1). These studies were of local (Sydney Harbour), regional (eastern Canada) and international scale to better understand the relevance of this study and the potential management implications for Sydney Harbour. All chemical contaminants measured in mussels in this study were lower than previously reported in mussels and other biota in Sydney Harbour (Hilderbrand 1982; Ernst et al. 1999; King and Chou 2003), elsewhere in eastern Canada (Fraser et al. 2011), and in mussels from the Gulf of Maine and elsewhere in the United States (O'Connor et al. 1998; McCullough et al. 2005).

These mussel bioindicators did not reveal negative impacts in Sydney Harbour resulting from remediation activities at the STP site. These findings were also corroborated in the results of other sampled ecological media, such as a lack of chemical signature in monthly water quality analysis and annual benthic community assessments (Dillon 2013). While current sediment contaminant concentrations are much lower than previously reported (Walker et al. 2013b), most of the contaminant inventory remains intact below the sediment surface, with the potential risk for future contaminant disturbance or resuspension (Grant et al. 2013). The lack of detection of PAHs and PCBs in mussels during most of the baseline and remediation activities would suggest that environmental management and mitigation protection measures at the STP site have been effective from the perspective of reducing significant environmental impacts on the marine environment (AMEC 2005). Contaminants are known to bioaccumulate in mussels (Chou et al. 2003), but the lack of detection of PAHs and PCBs in mussels in Sydney Harbour indicates how low contaminant concentrations are in the water column. This was corroborated by Dillon (2013), who reported that contaminants in water samples were mostly below detection limits. The natural recovery of Sydney Harbour sediments documented by Smith et al. (2009) and Walker et al. (2013b), coupled with environmental protection measures such as sediment and erosion control at the STP remediation site, has contributed to an overall reduction in the contaminant flux into Sydney Harbour.

The annual monitoring of chemicals in mussels in Sydney Harbour has not detected evidence of substantial contaminant releases into the marine environment despite remediation activities at the STP site. Measured metal concentrations in mussels, albeit low, showed some minor temporal variability (four years), but these did not appear to be directly related to remediation activities. None of the chemical parameters detected showed obvious signals around near-field stations compared to reference stations. For example, the highest concentrations of arsenic (As) and zinc (Zn) were measured at stations and assessment areas furthest away from remediation. Using mussels as bioindicators, the lack of detection of most individual PAHs and PCBs and low bioaccumulation of metals during baseline and remediation reveals subtle improvements in environmental quality in Sydney Harbour. Overall, chemical concentrations measured in this study were much lower than previously reported in other studies of mussels in industrial harbours in eastern Canada presumably due to the ongoing environmental recovery of Sydney Harbour and, to a lesser extent, to mitigation measures implemented during remediation. No obvious negative remediation impacts were detected during the study, but some perturbations were likely due to biological impacts (invasive tunicates) or harbour activities (shipping or dredging). Mussels proved effective as sensitive bioindicators, confirming EIS predictions of minimal impacts to the Sydney Harbour marine ecosystem.

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Table 1. Comparison of contaminant concentrations in mussels with other studies ($\mu g \cdot g^{-1}$)

Location	PAH	PCB	As	Cd	Cu	Hg	Pb	Zn	Reference
*CFIA Guideline Limit (2011)	–	2	3.5	–	–	0.5	0.5	–	CFIA (2011)
Sydney Harbour, NS, Canada (60 day)	<0.05-0.38	<0.05-0.07**	1.5-3.9	0.15-0.24	0.8-1.9	<0.01-0.03	<0.18-0.43	10-24	Present study
Sydney Harbour, NS (over-winter)	<0.05-0.27	<0.05-0.07**	1.9-3.2	0.14-0.29	1.3-1.7	<0.01-0.02	<0.18-0.37	13-22	Present study
Sydney Harbour, NS, (pre-deployment)	<0.05	<0.05	1.2-2.5	0.14-0.29	0.8-1.5	<0.01-0.01	0.22-0.63	11-26	Present study
Sydney Harbour, NS	0.78-5.00	–	–	–	–	–	–	–	Ernst et al. (1999)
Halifax Harbour, NS	–	–	1.9-2.5	0.10-0.44	1.5-2.4	0.01-0.04	0.1-2.4	17-41	McCullough *et al.* (2005)
Isaacs and Country Harbour, NS	<0.05	–	1.3-2	0.16-0.19	0.8-6.7	0.02-0.05	0.15-1.31	7-11	Dillon (2008)
Baie des Chaleurs, NB, Canada	–	–	–	0.55-4.2	0.5-1.1	–	<2.5-31	4.8-42	Fraser et al. (2011)
Gulf of Maine, 1994, United States	0.12-1.10	0.05-0.90	–	1.10-1.31	4.5-9.3	0.11-1.31	1.0-8.3	54-153	McCullough et al. (2005)
Gulf of Maine, 2009, United States	0.04-0.15	0.01-0.04	–	0.10-0.20	0.7-1.3	–	0.08-0.78	7-13	GOMC (2013)
Mussel Watch, United States (1986–1996)	0.19-0.50	0.06-0.15	8.3-10.1	1.90-3.20	8.6-10.0	0.09-0.12	0.6-1.1	120-143	*O'Connor et al. (*1998) (dw)

All tissue data based on wet weight (ww) unless otherwise indicated
dw = dry weight
* Canadian Guidelines for Chemical Contaminants and Toxins in Fish and Fish Products. Canadian Food Inspection Agency (2011)
** PCBs were <DL (<0.05 $\mu g \cdot g^{-1}$) during baseline, year 1 and 3, except for detections in some near-field stations (1-1, 1-2 and 1-4) during year 2

Figure 1. Concentration of fluoranthene in mussels and water in Sydney Harbour. Dashed horizontal lines indicate DLs (mussels = 0.05 $\mu g \cdot g^{-1}$; water = 0.01 $\mu g \cdot L^{-1}$). Sampling stations are centred on 1-4 near Muggah Creek (MC) and reference stations (R) are located furthest away (Adapted from Walker and MacAskill 2013).

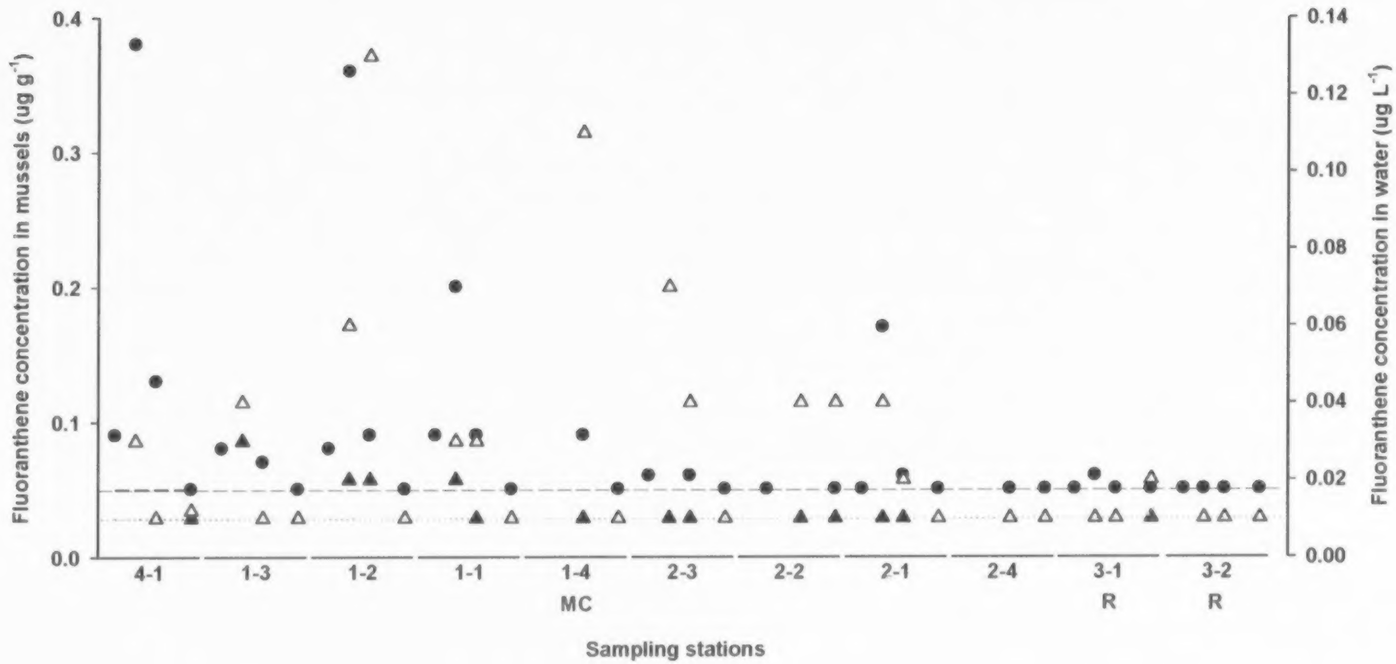
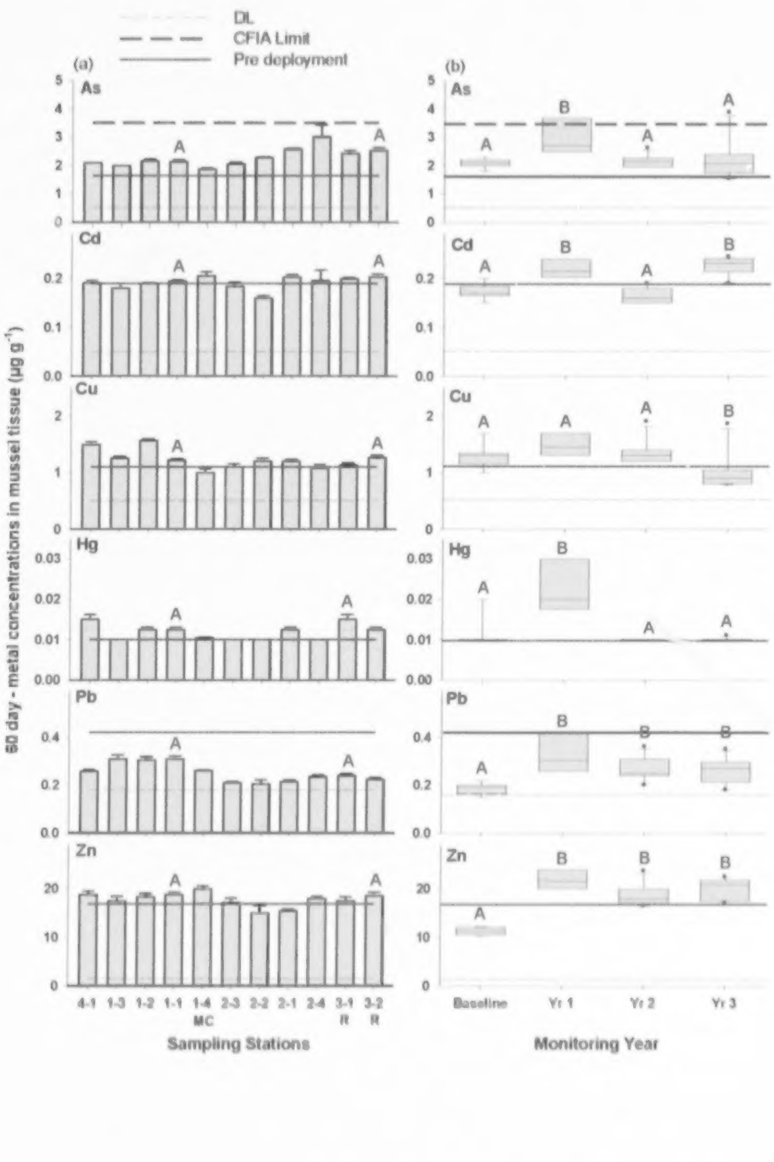

Fluoranthene concentration in mussels (ug g-1)
Fluoranthene concentration in water (ug L-1)
0.0
0.1
0.2
0.3
0.4
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
4-1
1-3
1-2
1-1
1-4
2-3
2-2
2-1
2-4
3-1
3-2
MC
R
R
Sampling stations

Figure 2. (a) Concentration of metals in mussels deployed for 60 days during baseline and three years of remediation. Dashed horizontal lines indicate DLs (As and Cu = 0.5; Cd = 0.05; Hg = 0.01; Pb = 0.18; Zn = 1.5 $\mu g \cdot g^{-1}$). CFIA guideline limit for As = 3.5 $\mu g \cdot g^{-1}$ (bold long dashed horizontal line). Solid horizontal line indicates mean pre-deployment concentrations for mussels (n = 6). Muggah Creek (MC) and reference stations (R) are indicated. (b) Box plots of metal concentrations showing temporal variation during baseline and remediation. Significant differences were determined by one-way ANOVA followed by Tukey's test; stations or years attributed with the same letters were not significant and those with different letters were significantly different at the $p < 0.05$ level. Hg pre-deployment concentrations and DL were the same (0.01 $\mu g \cdot g^{-1}$). CFIA limits for Hg (0.5 $\mu g \cdot g^{-1}$) are not shown due to axis scale. (Adapted from Walker and MacAskill 2013).



The co-dependency of growth and reproduction: A closer look at the 28-day *Leptocheirus plumulosis* survival, growth, and reproduction test (PL)

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The standard method for assessing chronic toxicity of sediments with a marine amphipod is a 28-day growth, survival, and reproduction test using *Leptocheirus plumulosis* as described in the U.S. Environmental Protection Agency (EPA) Method for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-associated Contaminants with the Amphipod *Leptocheirus plumulosus*, EPA 600/R-01/020, March 2001. This test is more expensive than standard growth and survival bioassays, and the reproductive endpoint often may not improve the sensitivity of the test. We performed a statistical analysis of control data from 12 separate studies. We found that there is a strong relationship between reaching a certain biomass and fecundity for individual organisms. When performing the bioassay, unless there is a certain benchmark met for growth in the controls, we do not see reproduction. Therefore, when evaluating organism biomass, one must ensure that adequate growth has been achieved in the controls and/or reference sediments before proceeding to assess the reproductive endpoint. Currently, there are only validity criteria for survival of the controls for this test. We propose that for the reproduction endpoint to provide useful data, a minimum growth threshold must be achieved in the controls.

Atlantic PIRI aquatic benchmarks for BTEX and petroleum hydrocarbons (PL)

R. Willis[1], U. Klee[2], R. Mroz[3], M. Stephenson[2], P. Miasek[4]
[1]Dillon Consulting, [2]Stantec, [3]Environment Canada, [4]Imperial Oil (retired)

Impacts to freshwater, estuarine, and marine water bodies from petroleum hydrocarbons are relatively common in Atlantic Canada (e.g., small craft harbours, current and former bulk plant sites, service station sites, fuel storage and handling sites, domestic oil spills). For years, there were few, if any, ground water, surface water and sediment benchmarks that could be applied in the assessment of hydrocarbon-impacted aquatic areas within Atlantic Canada. In July of 2012, Atlantic PIRI released groundwater, surface water, and sediment ecological screening levels (ESLs) for BTEX and petroleum hydrocarbon fractions. These ESLs allow for a more comprehensive evaluation of potential aquatic ecological risks due to petroleum hydrocarbons in freshwater, estuarine, and marine receiving environments. This presentation outlines the development of these ESLs, describes their application, and addresses follow-up work being undertaken to validate the ESLs.

Mercury concentration and biomagnification in a marine food web off southwestern Florida (PL)

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[1]University of New Brunswick, [2]Florida Gulf Coast University

Mercury (Hg) has received a lot of attention in southern Florida due to the high levels found in marine fish tissue that led to human consumption advisories. This study presents the results of a survey of both Hg and stable nitrogen isotopes (δ15N) in fish (40 species) and invertebrates (17 species) across a broad range of trophic positions to investigate Hg levels and biomagnification through a subtropical coastal food web. Twenty-five percent of the fish species sampled had average Hg levels exceeding consumption guidelines for the protection of human health. In general, observed Hg levels were often in the upper range of previously reported levels for other Gulf populations. Statistical comparisons done on size-normalized tissue concentrations in king mackerel (*Scomberomorus cavalla*) were significantly higher ($p < 0.001$) in the present study than Hg concentrations previously reported. Log Hg concentrations among species means were positively correlated with δ15N ($p < 0.001$, $R^2 = 0.66$). The $\log_{10}$[Hg]-δ15N slope, as a measure of Hg biomagnification through the entire food web, was 0.21 in this coastal food web when based on species means, and 0.27 when based on individual data points. A trophic magnification factor (TMF) of 5.05 was calculated from the relationship between log-transformed mean Hg concentrations and trophic level (calculated from δ15N), indicating that Hg increased by a factor of 5 with each increase in trophic level. The $\log_{10}$[Hg]-δ15N slope and TMF were also in the upper range of values reported from other regions and ecosystems.

Response of shrimp to sewage impact in the Saint John Harbour, Bay of Fundy, Canada (PO)

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[1]University of New Brunswick

The Saint John Harbour (SJH) estuary is one of six regional nodes in a Canadian Water Network-sponsored national project looking at developing monitoring programs in support of cumulative effects assessment. This study examines whether there are responses in sentinel species exposed to effluent from a new municipal wastewater treatment facility. Nearshore sampling was carried out at low tide, during the day, using a 9 m X 1.5 m beach seine with 9-mm stretch mesh. The most abundant epibenthic animal caught was sand shrimp (*Crangon septemspinosa*). The potential of sand shrimp as a sentinel species for contamination will be investigated. A good sentinel species is abundant, resident and demersal, and has easy-to-measure health endpoints. Sand shrimp from the sewage discharge site and four reference sites will be followed throughout an annual cycle to detect spatial and temporal changes in abundance, size, condition factor (length-at-weight), sex ratios and fecundity. Preliminary results show differences between sexes in condition factor (a metric of energy storage), size-at-maturity, and abundances. Females are more abundant than males (>70% of total catches) and have a higher condition factor and a smaller size-at-maturity at all sites. Stable isotope and contaminant body burden

analyses are in progress to help establish residence and movement patterns of shrimp caught at the different sites. If sand shrimp prove to be a good indicator of contamination, baseline biological data for the watershed will be developed. Determination of what is "normal" at multiple reference sites and optimal sampling times will provide a tool for watershed end-users to carry out cyclical monitoring in a dynamic marine environment that will see major developments in the years to come.

Environmental recovery in Sydney Harbour, Nova Scotia: Evidence of natural and anthropogenic sediment capping (PO)

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[1] Dillon Consulting, [2] Dillon Consulting / Cape Breton University, [3] Sydney Tar Ponds Agency

Abstract

Contaminants were assessed in Sydney Harbour during baseline and three years of remediation of a former coking and steel facility. Concentrations of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and lead (Pb) measured in surface sediments indicate that overall spatial distribution patterns of historical contaminants remain unchanged, although at much lower concentrations than previously reported due to natural sediment recovery. Recovery rates were in broad agreement with predicted concentrations or, in some cases, lower, despite remediation at the Sydney Tar Ponds (STP) site. Contaminants showed little temporal variability, except for detection of significant increases in PAH concentrations during onset of remediation compared to baseline, which represented a short-term interruption in the overall long-term natural recovery of sediments in Sydney Harbour. Recovery (via "capping") was enhanced following recent harbour dredging activities where less contaminated outer harbour sediments were discharged into a confined disposal facility (CDF) required for a new container in the inner harbour.

Introduction

Sydney Harbour, Nova Scotia, has long been subject to effluent and atmospheric inputs of contaminants, including thousands of tons of metals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) from a large coking and steel plant which operated in Sydney for nearly a century (Lambert et al. 2006). Contaminants were comprised of coal tar residues that were discharged from coking ovens into Coke Ovens Brook and a small tidal tributary, Muggah Creek, which in turn discharged into Sydney Harbour (Furinsky 2002). Numerous studies linked these effluent and atmospheric contaminants to local ecological and human health impacts (Tay et al. 2003), and these particle-reactive contaminants became the main pathway of exposure for marine benthic organisms (Stewart et al. 2002). Within Sydney Harbour, the highest chemical contaminant concentrations in sediments were historically reported near Muggah Creek, with concentrations decreasing towards the outer harbour (Lee et al. 2002).

The pollution legacy of contaminants in Sydney Harbour sediments is well documented. Contaminants reached their maxima between 1960 and 1980 (Lee et al. 2002). Smith et al. (2009) used radionuclide tracers to predict time required for natural recovery (or "capping") of historical

contaminants and estimated lead (Pb) concentrations would fall below the highest-ranked ecological health risk benchmarks (where chronic effects would be very likely) by 2020, and fall below lowest-ranked ecological health risk benchmarks (where chronic effects would be unlikely) by 2050. Concentrations of PAHs and PCBs were predicted to take a longer time for natural recovery to fall below their corresponding ecological benchmarks (2060 and 2090) and (2030 and 2060), respectively.

We measured current contaminant concentrations in Sydney Harbour sediments to compare against modeled predictions of natural sediment recovery rates reported by Smith et al. (2009). Monitoring occurred alongside two large-scale projects in or adjacent to the harbour: remediation of the Sydney Tar Ponds (STP) and large-scale dredging of less-contaminated outer harbour sediments, required for a new container terminal development in Sydney Harbour (Walker et al. 2013a, 2013b).

Annual monitoring of sediments began during 2009 baseline (pre-remediation) and continued during three years of remediation (year 1, 2010; year 2, 2011; year 3, 2012). Between October 2011 and December 2012 (monitoring years 2 and 3), nearly 5 million m^3 of cleaner sediment dredged from a navigation channel in the outer harbour was subsequently discharged into a confined disposal facility (CDF) located in the inner harbour.

Objectives of this study were: (1) to assess effectiveness of STP remedial activities on surface sediments in Sydney Harbour, (2) to measure potential positive impacts of anthropogenic sediment "capping" following dredging and discharge of less-contaminated sediments, and (3) to confirm modeled predictions of natural sediment recovery. A comprehensive suite of other monitored media and parameter concentrations have been reported elsewhere (Walker and MacAskill 2013; Walker et al. 2013a, 2013b, 2013c, 2013d).

Methods

Marine monitoring stations (~10 m deep) were established and sampled by boat during one baseline year and three years of remediation between September 2008 and August 2012. Stations were distributed across four assessment areas to reflect the harbour ecosystem, as follows: area 1 – near-field (four stations), area 2 – mid-field (four stations), area 3 – far-field (two stations), and area 4 – Sydney River Estuary (one station). These are described in greater detail in Walker et al. (2013a). Detailed methods of sediment sampling and chemical analysis can also be found in Walker et al. (2013b). Significant temporal differences between baseline and remediation (or following dredging) were determined by one-way ANOVA followed by Tukey's test; years attributed with same letters were not significant and those with different letters were significantly different ($p < 0.05$ level).

Baseline sampling data representing "before" were compared with subsequent annual monitoring events during remediation, representing "after" treatment. Concentrations of PAHs and PCBs were compared to sediment quality guidelines (SQGs) developed by the U.S. National Oceanographic and Atmospheric Administration (NOAA) for effects range-low (ER-L), corresponding to background concentrations below which presence of contaminants has little chronic or acute effect on benthic organisms, and effects range-medium (ER-M), above which organisms are very likely to be negatively affected by presence of contaminants (Buchman 2008). Lead concentrations were compared to Canadian Council of Ministers of the Environment (CCME) Canadian Water Quality Guidelines for the

protection of aquatic life probable effect levels (PEL) and the interim sediment quality guidelines (ISQG) (CCME 2007). Although ER-L and ER-M SQGs have been reported as overly conservative, they were used for comparison to previous modeled sediment concentration data by Smith et al. (2009) rather than toxicity of sediments *per se*, because CCME PEL and ISQG guidelines do not exist for total PAHs; they stipulate guidelines for individual PAH compounds instead. Toxicity of biota was assessed and discussed in separate studies (Walker and MacAskill 2013; Walker et al. 2013c, 2013d).

Results and Discussion

Generally, stations near Muggah Creek and Sydney River exhibited higher concentrations of contaminants, although these declined with increasing distance from Muggah Creek. Correspondingly, minimum concentrations were generally found in samples from far-field stations. Spatial distribution of contaminants was consistent with those of previous studies (Lee et al. 2002; Smith et al. 2009); however, maximum concentrations for PAHs, PCBs, and Pb were much lower.

Temporal variation in PAH concentrations was significantly different during year 1 remediation compared to 2009 baseline, but there were no significant differences between years 2 and 3 compared to baseline. Spatial variation in PAH concentrations decreased sharply with increasing distance from Muggah Creek, with the lowest concentrations (<5 $\mu g \cdot g^{-1}$) at far-field stations. Smith et al. (2009) predicted that, by 2020, surface concentrations of PAHs would still exceed ER-M near Muggah Creek, but our monitoring results reveal that most stations were lower than ER-M, although most stations still exceed ER-L values. However, some individual PAH compounds exceeded ISQGs (all stations) and PELs (some stations). Overall, ranges of PAH concentrations in surface sediments measured in Sydney Harbour between 2009 and 2012 (1.4-73.8 $\mu g \cdot g^{-1}$) were at least an order of magnitude lower than ranges determined by Smith et al. (2009) from geochrononologies of sediment cores representing concentrations from the 1960s-1980s (200-500 $\mu g \cdot g^{-1}$) (Table 1). Their study also predicted that PAH concentrations in surface sediments would eventually decrease to levels of 5-80 $\mu g \cdot g^{-1}$ by 2010 (comparable to ranges observed in our study) as a result of natural containment by less-contaminated sediments. Another study by Tay et al. (2003) in Sydney Harbour reported that PAH concentrations in sediments collected in 1999 were an order of magnitude higher (17-246 $\mu g \cdot g^{-1}$) than those measured in our study. Although the specific causes for significant increases in PAH concentrations in sediments during year 1 of remediation compared to baseline remain uncertain, absolute concentrations are low compared to historic levels. This slight increase represents a short-term interruption in the overall natural recovery of sediments in Sydney Harbour (Smith et al. 2009; Walker et al. 2013a, 2013b).

Prior to dredging, measured sediment deposition rates in bottom-moored traps were low in all areas (0.4-0.8 $cm \cdot yr^{-1}$) (Walker et al. 2013a). However, sedimentation rates measured during dredging operations were equivalent to 26-128 $cm \cdot yr^{-1}$ at mid-field stations, representing huge quantities of cleaner, coarser sediment for "capping" (Walker et al. 2013b). Smith et al. (2009) predicted that PAH concentrations would fall below ER-L in the central harbour by 2060. Encouragingly, all stations were currently below ER-M levels following dredging and "capping", but most stations still exceed ER-L levels.

There were no significant differences in PCB concentrations between baseline and each year of remediation. All stations currently exceeded ER-L values. Smith et al. (2009) reported that PCB levels in

sediments have increased since 1900, from <0.005 $\mu g \cdot g^{-1}$ up to maximum values of >1 $\mu g \cdot g^{-1}$ during peak activity at the coking facility in the 1960s-1980s (Table 1). Tay et al. (2003) also reported a maximum value of 3.4 $\mu g \cdot g^{-1}$ near Muggah Creek, comparable to the highest concentration measured in this study in area 1. Smith et al. (2009) predicted that PCB levels would fall below ER-M (0.18 $\mu g \cdot g^{-1}$) in the central harbour by ~2030. Our results indicate that whilst area 1 and 4 stations exceed this value, all stations in areas 2 and 3 were less than ER-M.

Sampling over four years showed only minor variation with no significant differences in sediment Pb concentrations, suggesting that recent inputs, as a result of remediation activities at the STP, were not detected above measured baseline conditions. The ISQG concentration for Pb (30.2 $\mu g \cdot g^{-1}$) was exceeded at most stations, and PEL (112 $\mu g \cdot g^{-1}$) was exceeded at some near-field stations. Smith et al. (2009) predicted that Pb concentrations would fall below ER-M (212 $\mu g \cdot g^{-1}$) and ER-L (46.7 $\mu g \cdot g^{-1}$) by 2020 and 2050, respectively, whereas current measurements indicate that most harbour stations were now lower than ER-M. Conversely, most stations exceed ER-L levels, where greatest effects of dredge discharge "capping" occurred near the CDF. These results confirm that Pb contamination of Sydney Harbour sediments does not pose a long-term threat and that natural containment will reduce Pb levels below those predicted to have a significant impact on organisms during the next 10-20 years (or possibly sooner if natural sediment deposition occurs uninterrupted). Despite these results, Pb concentrations in Sydney Harbour are currently lower than those measured in previous studies in the harbour (Table 1) and are below background concentrations reported for coastal sediments in Nova Scotia (Loring et al. 1996).

Ongoing natural recovery of sediments may have been anthropogenically enhanced following recent channel and berth dredging activities during years 2 and 3. Dredging of nearly 5 million m^3 of cleaner outer-harbour sediments from a 9-km navigation channel was required for a new marine container terminal in Sydney Harbour. Dredging, in combination with stringent sediment and erosion control mitigation at the STP site, may have enhanced contaminant "capping" of historic contaminants. These types of dredging and infilling operations normally result in major negative sediment disturbances such as resuspension, remobilization, and enhanced bioavailability of historical contaminants.

Whilst sediment contaminant concentrations are lower than those previously reported, most of the contaminant inventory remains intact below the sediment surface, with the potential risk for future contaminant disturbance or resuspension. Sediment stability and biological active zone are often affected by many factors, such as porosity, organic content, grain size, and bioturbation (Nedwell and Walker 1995; Walker et al. 2008). Therefore, quantifying sediment stability in coastal systems becomes an important predictive tool in order to understand fluxes of organic matter, particulates, and their associated contaminants (Walker and Grant 2009; Grant et al. 2013). The greatest risk of potential sediment erosion would be expected during high-energy conditions, including storms, flood events, and human disturbances—especially ship wake from cruise ships, which can be significant in Sydney Harbour.

Monitoring of PAH, PCB, and Pb concentrations in surface sediments in Sydney Harbour has revealed that current levels are in broad agreement with modeled predictions made by Smith et al. (2009). Attenuation of these contaminants has largely been the result of natural containment by layers

of cleaner surficial sediments from Sydney River. Furthermore, these natural recovery processes have occurred despite the large-scale remediation project at the adjacent STP site. Although concentrations of PAH, PCB, and Pb exceed the highest ecological bench marks (PELs or ER-M) at some near-field stations, absolute concentrations were low compared to historic levels. Assuming remediation demobilizes future releases of contaminants into Sydney Harbour sediments, recovery will continue at predicted rates or possibly faster, based on these results. The continuing trend of natural sediment containment in area 2 stations, and to a lesser extent in area 1, was also positively enhanced as a result of recent dredging activities in Sydney Harbour. Further monitoring of harbour sediments will be critical in order to measure rates of natural or anthropogenic sediment recovery.

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Table 1. Comparison of contaminant concentrations in sediments with other studies ($\mu g \cdot g^{-1}$). (Adapted from Walker et al. 2013b).

Location	PAH	PCB	Pb	Reference
Sydney Harbour, NS (2009-2012)	1.4-73.8	0.01-1.3	20-210	Present study
Sydney Harbour, NS (predicted 2010)	5-80	0.01-2.0	11-200	Smith et al. (2009)
Sydney Harbour, NS (peak 1960s-1980s)	200-500	1.0-6.0	100-400	Smith et al. (2009)
Sydney Harbour, NS	17-246	0.01-3.4	-	Tay et al. (2003)
Sydney Harbour, NS	-	0.19-190	43.9-584	JDAC (2001)
Sydney Harbour, NS (1999)	1-200+	-	50-300	Lee et al. (2002)
ER-L	4.02	0.023	-	Buchman (2008)
ER-M	44.8	0.18	-	Buchman (2008)
ISQG	-	-	30.2	CCME (2007)
PEL	-	-	112	CCME (2007)

Mercury in marine predators of Cape Shirreff (Livingstone Island, Southern Shetlands) and Paradise Cove (Antarctic Peninsula) (PO)

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Marine top predators are useful as sentinel species of mercury (Hg) pollution in non-industrialized coastal environments. In Antarctica, the Southern Shetland Islands and the Antarctic Peninsula are isolated coastal environments with very simple food webs. As the literature has shown in the past four decades, the diet of top predators influences their mercury concentrations. Due to the conservation status of the Antarctic biota, feces, feathers, and skin biopsies are a valid alternative to assessing Hg concentrations because they: (1) are a non-lethal sampling approach; (2) reflect the food source for chick(s) and mating penguins (Spheniscidae) and from individual leopard seal (*Hydrurga leptonyx*) at a given time; and (3) reflect the difference in incorporated and excreted Hg. The influence of the diet on the observed contaminant concentration was further investigated by stable isotope (δ13C, δ15N) analysis. The preliminary data showed a range in total Hg (THg) in biota from Cape Shirreff: 0.057-0.180 mg·kg^{-1} (dry weight [dw], penguin feces), 1.123-2.271 mg·kg^{-1} (dw, leopard seals feces) and 1.855-3.219 mg·kg^{-1} (dw, skua [*Stercorarius* spp.] feathers). In Paradise Cove, THg ranged in penguin feces 0.033-0.102 mg·kg^{-1} (dw) and skua feathers 1.617-3.685 mg·kg^{-1} (dw). Despite the fact that these are preliminary data, THg concentrations were highly and positively related to δ13C (R^2 = 0.931, Cape Shirreff; R^2 = 0.869, Paradise Cove) and positively related to δ15N (R^2 = 0.188, Cape Shirreff; R^2 = 0.257, Paradise Cove), highlighting the occurrence of efficient Hg biomagnification processes within marine trophic webs in the coasts of Livingstone Island and Antarctic Peninsula.

Effects of sediment contamination on benthic invertebrates in the Saint John Harbour (PO)

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Human activity around Saint John Harbour (SJH), particularly boat traffic and industries located near the harbour, provides several sources for metals, polycyclic aromatic hydrocarbons (PAHs) and other contaminants to enter the ocean. Upon entering the water, these pollutants are retained by sediment and accumulate over time, so there is a high exposure potential for sediment-dwelling organisms. This study investigates the impact of sediment contamination on benthic invertebrates in the SJH using a Sediment Quality Triad, an approach that combines chemical analysis and bioassays with impacts on benthic infauna. In particular, the components included in this Triad are analysis of metal and PAH content for sediment from each site, laboratory-run sediment bioassays, and changes in growth, abundance and fecundity of two benthic invertebrate species, the Atlantic nut clam (*Nucula proxima*) and a catworm (*Nephtys incise*). Samples were collected in October 2012, consisting of sediment grabs from four reference sites and three potential "hotspots" within SJH. Sites were selected

based on contaminant data collected previously by Environment Canada. Sediment grabs ($n = 5$ per site) were split for each analysis; one sample was sieved to retrieve animals for population effects, one was analyzed for metal and PAH content using inductively coupled plasma atomic emission spectroscopy and gas chromatography-mass spectrometry, and one was used for a suite of bioassays: amphipod toxicity, polychaete toxicity and growth, echinoid embryo development and a microtox assay. The sediment chemistry values indicate that the hotspots had few differences from the reference sites. Metal concentrations (lead, zinc and copper) exceeded sediment quality guidelines at only one site, and PAH concentrations did not differ greatly between reference sites and hotspots (total PAH content was 0.08-0.25 $\mu g \cdot g^{-1}$ at the hotpots and 0.12-0.20 $\mu g \cdot g^{-1}$ at the reference sites). Bioassay results indicated low toxicity for each site. Mean shell size in *N. proxima* does not appear to differ between sites (average shell length ranges 1.4-2.0 mm). Abundance and size-frequency distributions in *N. proxima* do differ between sites; however, the differences between reference and contaminated sites may not be significant. In summary, little evidence was found of elevated contaminant concentrations in the SJH and, concurrently, few effects on resident biota or those exposed to sediments in lab bioassays were identified.

Expression of metallothionein in white sturgeon (*Acipenser transmontanus*) following *in vitro* and chronic exposure to cadmium or copper (PO)

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White sturgeon (WS; *Acipenser transmontanus*) in the Columbia River (British Columbia, Canada) have been experiencing poor recruitment for over three decades. There are many possible causes for this phenomenon, including habitat alterations, genetic bottlenecks, predation by introduced species, and pollution (e.g., metals released by industrial and municipal sources). Acute and chronic exposure studies indicate that WS may be among the most sensitive species of fishes to certain metals, especially during certain early life stages. The purpose of this study was to address potential uncertainties associated with the mechanism of sensitivity of WS to metals such as cadmium (Cd) and copper (Cu). To this end, gene expression of metallothioneins (MTs), which are low-molecular-weight proteins that are important for the detoxification of various transition metals, was quantified in WS exposed to Cu or Cd. An MT gene encoding a protein of 64 amino acids was identified in WS. This MT has greatest homology to the MT-4 of mammals. Fishes studied to date are known to express MT-1 and MT-2 genes, while higher vertebrates are known to express MT-1, MT-2, MT-3, and MT-4. This is the first report of an MT-4 being expressed in fish and might be a trait unique to ancient species of fishes, such as sturgeons. No significant alterations in abundances of transcripts of MT-4 were quantified in gills or livers from WS exposed from embryos for 19 or 58 days to Cu (0, 1.8, 5.9, 36 $\mu g \cdot L^{-1}$) or Cd (0, 0.17, 1.1, 8.3 $\mu g \cdot L^{-1}$). An *in vitro* liver slice assay using juvenile WS was used to investigate acute effects of Cu or Cd to MT-4. No significant trend in alterations in transcript abundance of MT-4 was observed following exposure to Cd for as long as 24 hours. However, a significant down-regulation was observed after 8 hours of exposure to 0.3, 3, 30 or 100 µM of Cu. Therefore, the MT-4 in WS might not provide a mechanism of detoxification of Cu or Cd and might represent one reason for sensitivity of WS to these

metals. Research is currently ongoing to identify whether additional MT genes are expressed in WS, particularly MT-1 or MT-2 genes that are known to be responsive to metals in other species of fishes.

Use of vitellogenin gene expression in the assessment of the sensitivity of four native Canadian fish species to 17α-ethinylestradiol, *in vitro* (PO)

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Exposure to environmental estrogens and other endocrine-active chemicals have been shown to impact reproduction of freshwater fish species. One environmental estrogen of particular concern is the synthetic estrogen 17α-ethinylestradiol (EE2), which has 10- to 50-fold greater potency than natural estrogens. While extensive research has been conducted regarding the interaction of this chemical with model laboratory and some European fish species, little is known about the potential effects of environmental estrogens to freshwater fish species native to North American environments. Conducting standard *in vivo* toxicity tests with fish requires large numbers of animals, which has become an increasing concern from an ethical perspective, especially when working with protected species. Therefore, the aim of this present study was to investigate the sensitivity to EE2 of four fish species native to Canadian freshwater ecosystems, using an *in vitro* liver explant assay. Transcript abundances of vitellogenin (VTG), the gene that encodes for a precursor of egg-yolk protein, was chosen as the endpoint in this study, as it is a highly sensitive biomarker used to assess exposure to environmental estrogens. Specifically, liver tissue of northern pike (*Esox lucius*), walleye (*Sander vitreus*), white sucker (*Catostomus commeroni*) and white sturgeon (*Acipenser transmontanus*) were excised, sliced into 1-mm^3 pieces and placed into a 24-well cell culture plate containing L-15 media. The liver tissue was exposed to a solvent control (DMSO) and seven concentrations of EE2 (3, 10, 30, 100, 300, 1000, 3000 ng $EE2 \cdot L^{-1}$). Twelve-, 24- and 48-hour exposures were completed to identify the optimal exposure time for each species. Preliminary results showed a time-dependent increase in transcript abundance of VTG in white sucker males, with a 48-hour exposure to EE2 resulting in the greatest concentration-dependent increase. No increase in transcript abundance of VTG in white sturgeon liver between 24- and 48-hour exposure times was found. However, the 24-hour exposure time-point was less variable, deeming it optimal. Of the two species currently tested, white sucker was found to be more sensitive to EE2 compared to white sturgeon, with the LOECs of 10 ng $EE2 \cdot L^{-1}$ and 300 ng $EE2 \cdot L^{-1}$, respectively. Ongoing analysis of the transcript abundances of exposed livers of northern pike and walleye, along with the transcript abundances of estrogen receptors ER-α and ER-β, are underway.

Atlantic Reference Centre: A microcosm of ocean biodiversity informatics standards (PO)

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The process leading to the sharing of biological data is based on specimens and standards. Biological data are obtained through the costly collection of specimens. The value of these expensive specimens will be maximized through the use of authoritative standards in each step of the data-sharing process following collection: identification, taxonomy, archiving, digitizing and transformation to formats for sharing via standardized metadata and portals. These standardized data can be used in-house or by others for research or to develop products, such as regional species lists and species information systems. The Atlantic Reference Centre (ARC) is a museum of Canadian Atlantic organisms and a principal repository for specimens collected by Fisheries and Oceans Canada. The ARC museum database is a source of biodiversity information to be shared, and is the basis of biodiversity information products. Therefore, the ARC is an institution that practices all stages of the data-sharing process based on specimens and standards. Some of the pitfalls encountered and lessons learned involve taxonomic standards, data quality, data transformability, and the importance of accurate metadata.

Onshore Oil & Gas

Oil spills in freshwater: Why we should be concerned (PL)

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The traditional image of an oil spill involves ships or oil rigs sinking, waves crashing, sticky shorelines and wildlife destruction in marine ecosystems, as exemplified by the Exxon Valdez and Deepwater Horizon incidents. However, major oil spills at sea account for only a small percentage of the total spilled annually; more than 50% is spilled on land because of a high frequency of small accidents due to transportation, production, refining and use of oil and oil products. The volumes of oil in freshwater often exceed 1 million litres, but are generally much smaller than in marine ecosystems. Nevertheless, the consequences can be far more serious and long lasting, as exemplified by spills to the Kalamazoo River, Michigan (2010), and the Pine River, British Columbia (2005). Features that are unique to freshwater systems include a high ratio of river banks to water mass, lower dilution ratios, the propensity of oil and diluted bitumen to sink in fresh water, the greater capacity of surface oil to interact with sediments due to turbulence in shallow waters, the potential for hyporheic flows to transfer oil to spawning shoals where bed sediments are highly porous, contamination of riparian lands during floods, and habitat damage associated with clean-up. The overall impact of these differences is a great deal of uncertainty in defining short- and long-term impacts, and a much higher clean-up cost per litre of spilled oil.

Larval fish toxicity of sediments, waters, groundwaters and snowmelt waters from oil sands areas (PL)

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In support of developing a more comprehensive picture of the toxicology of natural and oil sands-related environmental samples, a series of laboratory fish tests was conducted. One of the goals of the toxicity tests was to examine pathways and sources of contaminants that may be causing effects in wild fish and invertebrates. Samples were collected from sites where wild fish health assessments and invertebrate communities were assessed in the oil sands area. In this way, linkages could be made between wild invertebrates and fish, in comparison to controlled studies of lab fish exposed to certain components of the environment (sediment, water, groundwater and snowmelt). Embryo-larval fathead minnows were used to assess the chronic toxicity of the following environmental samples: river sediments, river waters, groundwaters, snowmelt waters, spring freshet waters and suspended sediments collected near oil sands processing facilities along the Athabasca River and tributaries in areas

of oil sands development. These were compared to samples collected far from sites of oil sands mining and processing. Fertilized fathead minnow eggs were exposed for 21 days to samples in dose-response gradients. The majority of the environmental samples caused no effects in larval fish in 21-day assays. A limited number of samples caused effects in larval fathead minnows: snowmelt samples, groundwaters and sediments from the Steepbank and Ells Rivers, and waters from the Muskeg River. Some of the samples causing effects were from sites close to industrial activity (Steepbank River sediments, snow samples close to stacks, Muskeg River waters). However, other samples (groundwaters, Ells River waters) that caused effects were collected far from oil sands activities, at "background" sites where natural oil sands weathering or water movement through bitumen occurs. Samples were analyzed for naphthenic acids, polycyclic aromatic hydrocarbons (PAHs), C1-C4 alkylated PAHs, and metals. Sites where sediments and waters caused effects in lab fish bioassays are being assessed to determine whether wild young-of-year fish are abundant and growing normally in these areas. These studies are contributing to our understanding of regional and local patterns, and show the challenges of separating anthropogenic versus natural input in the oil sands region. The results of this work will help guide future studies and locations in sampling wild fish and invertebrates to fully assess environmental health in the oil sands area.

Plasma protein profiles of white and longnose sucker (*Catostomus commersonii* and *C. catostomus*) sampled from the Athabasca River, upstream and downstream of oil sands development (PL)

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Oil extraction from the bitumen deposits in the Athabasca Oil Sands region in northern Alberta generates large volumes of contaminant waste in the form of tailings and oil sands process-affected waters (OSPW). There are questions about whether OSPW or their components might enter the Athabasca River, which drains into the Peace-Athabasca Delta, at concentrations sufficient to cause effects in the biota. Shotgun proteomics can be used to assess the health of animals to determine protein biomarkers that are specific to environmental exposures, and also to characterize unique mechanisms of action of contaminants. As part of a larger wild fish health assessment, we have successfully developed and applied shotgun proteomics to generate protein profiles from plasma of mature male and female white sucker taken from the various sites along the main stem of the Athabasca River in 2011 and 2012. The study sites were located within and outside of the oil sand deposit, including a site downstream of Fort McMurray but above the oil sands operations, and two sites downstream of the oil sands extraction facilities. Over 6,000 proteins were identified across all sampling locations for both years. On average, 376 ± 96 proteins were identified in plasma from each location in both years. Gene names corresponding to those identified proteins were analyzed using interactive pathway software (Ingenuity Systems, Inc.) to determine their core functions and to compare the datasets by location, year, and sex. There were over 500 unique proteins expressed only in fish sampled from the location that was downstream of the oils sands development. Those unique proteins

were significantly ($p < 0.01$) related to neurological disease, skeletal and muscular disorders, and development disorders. The data suggest that wild fish residing downstream of oil sands operations may be exhibiting unique, site-specific responses in terms of their plasma protein profiles compared to fish residing upstream of oil sands operations. The proteomics data will be compared to a suite of health metrics for the sampled fish to assess concordance, to understand mechanisms of potential health effects, or to suggest additional health metrics for assessment where applicable.

Biliary naphthenic acids as chemical indicators of exposure to oil sands-affected waters (PL)

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Naphthenic acids are known to be one of the most prevalent groups of organic compounds in oil sands tailings-associated waters, and their biliary metabolites may serve as indicators of exposure to water influenced by the oil sands-derived naphthenic acids. Several methods of bile metabolite analysis were compared in yellow perch (*Perca flavescens*) either exposed for four months to oil sands-influenced waters in two experimental systems, or collected from three lakes at varying distances from oil sands mining activity. Bile metabolites were measured using gas chromatography-mass spectrometry (GC-MS) techniques, high- performance liquid chromatography (HPLC) with fluorescence detection at phenanthrene wavelengths, and with high-resolution mass spectrometry detection (LC-HRMS). Bile fluorescence and LC-HRMS methods were capable of statistically distinguishing samples originating from oil sands-influenced waters versus reference lakes. Although the concentrations measured with the GC-MS and HPLC fluorescence methods were correlated, there were no significant correlations between those methods and the LC-HRMS method. LC-HRMS was shown to be a highly sensitive and selective technique for determining the exposure of biota to oil sands-derived naphthenic acids. This technique was applied to the bile of white sucker collected in the Athabasca River near oil sands activities and from a reference lake located off of the oil sands deposits. Bile was analyzed by HPLC fluorescence techniques and by LC-HRMS using the mass-to-charge (m/z) ratios of seven isomer groups found to be selective for oil sands exposure in the perch study. These isomers were quantified against a naphthenic acid preparation purified from oil sands tailings water. Though there were differences in the patterns of biliary naphthenic acid metabolites as compared to perch exposed to tailings-associated waters, overall, sucker bile showed a clear signal of exposure, with five of the seven ions occurring in the bile of Athabasca River sucker. Bile fluorescence also showed an increase in exposure to aromatic compounds in the oil sands-exposed sucker.

Toxicity of oil sands naphthenic acids to early life stages of fathead minnows and walleye (PL)

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Naphthenic acids (NAs) are present in both natural waters and oil sands process-affected water (OSPW) in northern Alberta's oil sands regions. In this study, we report on NAs extracted from oil sands process waters, and compare them to a commercial extract. Fathead minnow (*Pimephales promelas*) and walleye (*Sander vitreus*) embryo-larval survival and growth were studied after exposure to NA extracts (NAE) generated from OSPW collected in 2009 and 2011, as well as to commercially obtained NAs. Oil sands-related NAs were extracted from OSPW from the Athabasca oil sands area. Fathead minnow embryos less than 24 hours old were reared in NAE ranging from 0 to 40 $mg \cdot L^{-1}$ through to either hatch or 15 days post-hatch (dph) for a 21-day exposure. Walleye embryos less than 48 hours old were reared in NAE ranging from 0 to 40 $mg \cdot L^{-1}$ to 7 dph for a 20- to 22-day exposure. Larvae of both species were assessed for deformities and effects on growth. In both species, the egg stage appeared to be more sensitive than the larval stage; NAE delayed hatch, sometimes decreased hatch length, and decreased hatch success for both species (LC_{50} = 10-20 $mg \cdot L^{-1}$). In fathead minnows, commercially sourced NAs (LC_{50} of 2 $mg \cdot L^{-1}$) were more toxic than NAE derived from OSPW. NAE also reduced spontaneous flexures and decreased heart rates in fathead minnow embryos 1-2 days post-fertilization (dpf) at exposures comparable to the at-hatch LC_{50} (5-6 dpf). Our work contributes to the understanding of naphthenic acid toxicity to multiple fish species during their vulnerable periods of early development, an important step in planning remediation for tailing ponds in the Athabasca oil sands region. This important baseline work contributes to our understanding of the role of these compounds in OSPW and in the natural environment.

In vitro assessment of endocrine-disrupting potential of naphthenic acid fractions from oil sands-influenced water (PL)

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Extraction of bitumen from oil sands produces large volumes of oil sands-influenced water, and there is concern surrounding the toxicity of its constituents, especially the water-soluble organic fraction referred to as naphthenic acids (NAs). The goal of this study was to use *in vitro* assays to determine if specific NA fractions obtained from oil sands-influenced waters possess biological activity. NAs were extracted in bulk from oil sands-influenced waters using acid precipitation. Basic aqueous extract solution was extracted with dichloromethane (DCM) to provide a neutral/hydrophobic fraction (DCM fraction). The remaining extract was re-precipitated at acid pH 2 and the pellet was washed and freeze-dried (main fraction). Compounds that did not precipitate were extracted using C-18 cartridge and eluded with either methanol (C18 MeOH fraction) or 1:1 MeOH:NaOH (C18 NAOH fraction), resulting in two additional fractions. The four fractions were examined for sex steroid receptor binding activity using

the yeast estrogen screen (YES) and yeast androgen screen (YAS). The chemical nature of the fractions was evaluated by high-resolution mass spectrometry (HRMS), 1H nuclear magnetic resonance (NMR) and attenuated total reflectance (ATR) infrared spectroscopy. None of the fractions contained measurable levels of estrogen or androgen receptor agonists. However, a number of fractions showed anti-estrogenicity and anti-androgenenicity, with levels being highest in the DCM fraction. Both anti-androgenic and anti-estrogenic receptor binding appear to be associated with the fraction most likely to contain neutral or hydrophobic compounds. The exact compound(s) responsible for these effects remains to be determined.

Athabasca mainstem operational long-term water quality monitoring (PO)

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The Joint Canada/Alberta Implementation Plan called for the establishment of new long-term water quality monitoring sites on the Lower Athabasca River mainstem as part of oil sands monitoring activities. To support the required contaminant loading approach as outlined in the Lower Athabasca Water Quality Monitoring Plan (Phase 1), U.S. Geological Survey flow-weighted sampling methods were adapted to the Athabasca mainstem. These methods can address the sand-bed braided nature of the Lower Athabasca River. In addition to the cross-channel water quality sample collection, cross-channel depth and velocity profiles are developed at each site, documenting how riverbed characteristics change over time. At the same time, traditional sampling methods are being used to retain a direct connection and comparability with historical data. Feasibility of passive sampling for polycyclic aromatic hydrocarbons (PAHs) using semi-permeable membrane devices (SPMDs) is under examination. Environment Canada, in partnership with Alberta Environment, is developing deployment techniques appropriate to the Lower Athabasca River, taking into account the large flow variability and high debris during high-flow events.

Effects of maternal dietary selenium exposure on early life stages of the amphibian *Xenopus laevis* (PO)

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Selenium (Se) is a contaminant of potential concern in aquatic systems due to its efficient incorporation into food webs and its role as a developmental toxicant in oviparous vertebrates such as fish and birds. Currently, there is a lack of Se toxicity data for amphibians, particularly regarding tissue-based thresholds in early life stages. The objective of this study was to determine dose-response relationships for early life stage toxicities in the model amphibian, *Xenopus laevis*. Following a 60-day dietary exposure to food augmented with L-selenomethionine (SeMet) at nominal concentrations of 0, 10, 30 and 90 µg Se$\cdot$g^{-1} dry mass, adult female *X. laevis* were bred with untreated males. Evaluated endpoints included egg Se concentration, fertilization success, hatchability, survivability, developmental

rates, and frequency of malformations in developing larvae. Preliminary results indicate that Se had no effect on fertilization success or adult survival in *X. laevis*, but is incorporated into eggs at concentrations that reflect adult dietary exposures. Further research will determine the frequency, severity, and type of malformations in tadpoles at 5 days post-fertilization and after completion of metamorphosis. Overall, this study will contribute to a better understanding of the sensitivity of amphibians to Se exposure.

Molecular and physiological effects of chronic dietary selenomethionine in juvenile white sturgeon (*Acipenser transmontanus*) (PO)

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Sturgeon are an ancient and unique family of fish. Of the 26 species worldwide, many are endangered. White sturgeon (*Acipenser transmontanus*), an anadromous species, are endemic to western North America, where they are prized by Aboriginal peoples and sport fishers. Unfortunately, their populations have been steadily declining since the late 1800s due to overharvesting, habitat alteration, and pollution. Selenomethionine (SeMet) has become a particular environmental concern as it is known to bioaccumulate and biomagnify through the food chain and is becoming prevalent in major water bodies around the world. Given their longevity and benthic lifestyle, sturgeon may be at particular risk of SeMet exposure. This study investigated the effects of chronic dietary SeMet on juvenile white sturgeon. Juvenile sturgeon were given food spiked with 0, 5, 25, or 100 ug SeMet$\cdot g^{-1}$ food for 72 days. Greater than 30% mortalities were observed in the high dose, as well as severe edema in the body cavity causing exopthalmus (popeye), in more than 20% of high-dose fish. Liver and head kidney deterioration, loss of muscle mass, and food aversion were observed in high-dose fish. Selenium concentrations in muscle tissue exceeded the common effect level of 3 $\mu g \cdot g^{-1}$ by more than 1.5 times in high-dose mortalities. No mortalities occurred in other treatment groups. A 10-day sub-sample was collected to investigate molecular changes across the transcriptome using the Illumina sequence-by-synthesis method to determine potential novel adverse outcome pathways. At this time, a few genes of interest, such as those involved in oxidative stress and the hypothalamus-pituitary-adrenal axis, have been verified using real-time PCR. Ongoing research will link molecular changes to observable whole-organism effects by investigating organ histopathology and alterations in stress response and oxidative stress pathways. This study aims to increase the understanding of white sturgeon sensitivity to SeMet and to aid in the risk assessment of this unique and endangered species.

Pulp & Paper Investigation of Cause

Pulp and paper investigation of cause and solution studies: A summary of results and description of common and novel approaches (PL)

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By the end of the sixth cycle of Environmental Effects Monitoring (EEM) required by the *Pulp and Paper Effluent Regulations*, most pulp and paper mills had completed Investigation of Cause (IOC) and Investigation of Solution (IOS) studies for confirmed effects observed in the aquatic receiving environment. Mills began conducting investigation studies in Cycle 4 (2004-2007) and the number of mills conducting these studies has increased since then, with the number expected to peak in Cycle 6 (2010-2013). The two most common types of effects under investigation were (1) reduced gonad size in fish and (2) effects associated with eutrophication. The primary purpose of most IOC studies for effects associated with eutrophication was to determine if the observed effects were caused, at least in part, by mill effluent as opposed to other nearby discharges. To sort out these confounding factors, various study designs and novel tools were used. To investigate the cause of reduced gonad in fish, most mills participated in a collaborative national research study, the purpose of which was to select the most appropriate laboratory tests for conducting investigation studies aimed at determining cause and identifying potential solutions for effluent-related effects on fish reproduction. IOS studies for effects associated with eutrophication focused on mill processes contributing to wastewater treatment plants and the management of the treatment plant itself. To identify possible solutions to reduced gonad size in fish, the national research study focused on the relationship between effluent parameters and egg-laying success in laboratory studies. The cause most commonly identified for effects associated with eutrophication was enrichment due to nutrients in mill effluent discharged to the receiving environment. The direct cause of reduced gonad size in fish remains unknown; however, a strong relationship between organics (biochemical oxygen demand) in mill effluent and egg-laying success in laboratory fish was established. Possible solutions identified for eutrophication-related effects and reduced gonad size in fish are both related to the continued improvement of effluent quality through improved mill processes and wastewater treatment. Over the next few cycles, mills will be reassessing effects and, depending on the degree to which mills choose to implement identified solutions, the overall impact of mill effluent on the aquatic receiving environment could be quite different.

Review of resemblance indices and statistical comparisons for use in the Environmental Effects Monitoring program (PL)

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Environment Canada recently contracted a review of resemblance indices and methods for statistical comparison, to evaluate their relative strengths and weaknesses for use in the Environmental Effects Monitoring (EEM) program. The particular focus of the review was to evaluate what has been called the "Bray-Curtis" endpoint, which is used to measure changes in community structure for benthic invertebrates exposed to industrial effluents such as those produced by pulp and paper mills and metal mines. Resemblance indices (Bray-Curtis and others) were evaluated based on factors such as sensitivity to measurement unit, usability in linear methods, correction for undersampling, frequency of usage, ease of computation, availability in statistical packages, and applicability to incidence (presence/absence) versus abundance data. Statistical tests were evaluated, in part, based on statistical power to detect effects that may be occurring and on minimizing false positives due to inaccurate estimates of Type 1 error. The best combination of good statistical power and accurate estimates of Type 1 error was found when using the Bray-Curtis dissimilarity index and statistically comparing reference to exposure areas using either the Mantel test or distance-based redundancy analysis (dbRDA) on transformed data. The implications of using different sample sizes were also investigated. The findings of this review have very little effect on the outcome for regulated facilities currently in the EEM program, so reanalysis by facilities of past cycles or phases of data is not needed. Environment Canada is committed to continuous improvement of EEM tools and will incorporate these new methodologies into its EEM Technical Guidance Documents for use in future EEM studies.

Progress update of the national Investigation of Cause/Investigation of Solution project for cost-effective solutions to reproductive effects in fish exposed to pulp and paper mill effluents (PL)

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The pulp and paper Environmental Effects Monitoring (EEM) program identified two main environmental issues related to mill effluents. In the case of benthic invertebrates, the evidence pointed to enrichment. In the case of fish, there was also evidence of enrichment and metabolic disruption, a term used to describe impacts on reproductive capacity, defined by the occurrence of larger fish with larger livers and proportionally smaller gonads. When effects are identified, the EEM program calls for Investigation of cause (IOC) and Investigation of Solution (IOS) studies. For fish, due to the complexity of the issue, a National Project involving the pooling of resources under a consortium was formed to tackle the challenge. Initial work during Cycle 4 focused on the selection of a reliable laboratory tool for

assessing the potential effects of mill effluents on fish reproduction. The evaluation of laboratory assays identified an adult fathead minnow reproduction test, with egg production as the main endpoint, as a promising tool for IOC and IOS studies. During Cycle 5, this test was applied to a study of effluent quality at a kraft mill. The results showed that control of organic losses is an important component of a strategy to abate effluent-related effects on reproduction. Measurement of biological oxygen demand (BOD) and gas chromatography-mass spectrometry (GC-MS) profiles were determined to be good indicators of organic losses due to spills, upset conditions or underperforming effluent biological treatment. In Cycle 6, these tools were applied to 20 mills across Canada. This provided an opportunity to assess the relationship between organic losses measured by chemical analysis of the effluents and the effects of effluents on fish reproduction. For the majority of mills participating in this study, reducing the loading of organics in the final effluent appears to provide the greatest probability of abating effects on the reproduction of fish in our laboratory tests. This study suggests that improvements in effluent quality are achievable through optimization of the biological treatment plant, spill minimization, and the reduction of BOD throughout the process. The test data provide strong evidence that the target level of 25 $mg \cdot L^{-1}$ residual BOD is robust and that the mill efforts to reduce their BOD below this level will result in improved effluent quality and the elimination of effects on fish reproduction in our test.

A history of Investigation of Cause for three mills in New Brunswick (PL)

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J.D. Irving operates three pulp/paper mills in southern New Brunswick: Irving Pulp and Paper (IPP) at Reversing Falls in Saint John, Irving Paper Limited (IPL) at Courtenay Bay in Saint John, and Lake Utopia Paper (LUP) near St. George. The IPP and IPL mills entered IOC in Cycle 4, based on results of mesocosm studies that suggested the potential for reproductive effects to fish. Field studies for fish for these two mills were fraught with challenges due to the highly dynamic and energetic receiving environment, industrial history in Saint John Harbour, and other sources of industrial and municipal inputs nearby. The LUP mill entered the Investigation of Cause (IOC) phase in Cycle 5 based on effects to fish observed in the field studies; this mill has also participated in the National IOC Project. The IOC studies are research-based and collaborative for the three mills, resulting in the development of optimized bioassays for reproduction in fish, insight into possible causative constituents, and new areas for investigation. This presentation will review the history of IOC for these three mills and identify current questions that contribute to future directions for these mills and also for consideration by the National IOC Project team and Environment Canada.

Teasing out historical from present-day effects: AV Cell pulp and paper mill Investigation of Cause (PL)

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The AV Cell mill in Atholville, New Brunswick, has operated at the present site since 1920. Previous Environmental Effects Monitoring (EEM) cycles identified effects to the near-field benthic macro-invertebrate community (BMIC). However, it was unclear if observed effects were the result of ongoing exposure to mill effluent or exposure to historically altered substrates. The Investigation of Cause (IOC) program for AV Cell was designed to discriminate between the effects of sediment type and effluent on the BMIC. The experimental design was a 2x2 factorial design analyzed using a 2-way ANOVA. Experimental pots containing bark or gravel substrate were deployed both in the near-field and in the far-field downstream of the effluent outfall. There was a significant enrichment effect of mill effluent on invertebrate density, and a significant inhibitory effect on benthic invertebrate diversity, evenness, and the Shannon-Weiner index. There was also an inhibitory effect of substrate type for all benthic invertebrate endpoints, though the observed differences were considerably less than those observed for the effect of effluent, and not always significant. Effects observed were therefore strongly related to ongoing effluent exposure, with substrate type having a secondary and weaker influence on the benthic invertebrate community.

Examination of legacy effects in a pulp mill effluent-receiving environment (PL)

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Pulp mill effluent has been a major global aquatic pollutant for decades. Despite the implementation of improved treatment and environmental practices, measurable effects can persist in exposed fishes. In some cases, it is unclear if this is due to present-day effluents or related to historical sediment contamination. The goal of this project is to investigate the hypothesis that a wild fish population in an area formerly receiving effluent may be exposed to legacy contaminants, resulting in alterations in population parameters. Brook or five-spined stickleback (*Culeas inconstans*) were sampled both from an effluent-receiving area and from nearby, unexposed reference site(s) in May 2007 (pre-mill closure) and May 2011. In the pulp mill effluent-exposed area, stickleback were extirpated, presumably due to anoxia, in summer 2011 and remained absent in spring 2012 and 2013. EROD analysis revealed that females from the effluent-receiving site had consistently higher CYP1A induction in 2007 and 2011, with the difference in 2011 being significant. Males showed less consistent patterns, with their induction being significantly reduced in 2007 and significantly amplified in 2011. Male liver somatic indices were consistently significantly higher in fish from the effluent-receiving area in both 2007 and 2011. Female fish shared this pattern, barring one reference site in 2011. Gonad somatic indices of females from the effluent-receiving site were significantly higher compared to reference sites in 2007 and 2011, again

barring one reference site in 2011. Female gonadal stages among all sites were significantly different from one another, and the effluent-receiving area fish were the second most mature of the four groups of fish. In 2011, effluent-receiving area males and females had significantly higher condition factors than those at reference sites. This was not the case in 2007. Temperature data show that areas with warmer temperatures tend to have fish with higher condition factors. Additionally, areas with warmer over-wintering temperatures had stickleback that were further along in their reproductive maturity. Chemical evaluation of sediment from the receiving environment shows elevated levels of resin acids and of the PAH retene. However, interpretation of the results was complicated by periodic opening and closing of this pulp mill during the experimental period.

Designing triggers for environmental monitoring using data from reference sites: Detecting recovery of fish after the closure of a bleached kraft pulp mill in northern Ontario (PL)

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An important and often implicit component in ecotoxicological studies is recovery of biota after a disturbance. A key variable in determining recovery is a defined target. Ideally, a long-term data set before the disturbance would be available, but this is rarely the case. In place of that ideal, many studies use upstream reference sites to define normal. Following the closure of a mill in 2006 we conducted follow-up studies to determine the performance of fish using measurements at an upstream reference site as the target for recovery. In contrast to the varying effects detected before the closure of the mill (i.e., reproductive impairment, nutrient enrichment), in 2011 we found a food-limited response in white sucker (*Catostomus commersonii*) augmented by low catch rates compared to fish from the upstream reference site. Collections repeated in 2012 found no differences in liver size, gonad size or condition, but the female fish had reduced fecundity and low relative abundance compared to upstream. Results from these two years of study suggest that the effluent was an important resource for the ecosystem in the downstream area and the population may be adjusting to the new carrying capacity of the system. However, the context of these changes is not certain. To provide context, we evaluated our results relative to regional data collected since 1991. These contextual analyses allowed us to evaluate regional changes over time and the ecological relevance of the data we observed after the closure of the mill, and to estimate the number of sites needed to approximate normal for a system. Within this analysis, we found that white sucker collected in the Mattagami River in 2011 and 2012 exhibit a temporal decline that is exacerbated downstream of the closed mill. Estimates of ecological relevance from the regional data show that declines in performance of some endpoints (GSI, K) are not unusual, while others are extreme (LSI) and may suggest persistent sensitivities in the ecosystem after the closure of the mill. We also found that little extra information on the normal performance of a fish population is gained by sampling more than 8-16 sites. In conclusion, we suggest that the information on impacts is greatly enhanced by the inclusion of long-term data. The long-term data can be used to track both

regional and local changes over time, and to develop critical effect sizes to evaluate the ecological significance of changes, which eludes many small and short-term studies.

Proteomic profiles of white sucker (*Catostomus commersonii)* sampled from the Jackfish Bay Area of Concern (PL)

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White sucker (*Catostomus commersonii*) sampled from the Jackfish Bay Area of Concern in Lake Superior were assessed using a shotgun approach to compile proteomic profiles. The Jackfish Bay Area of Concern is of interest because it has been impacted for many years by a pulp and paper mill. Mill activities were suspended in October 2010 and then resumed until October 2011, when the mill re-closed to re-open in October of 2012. Both male and female fish were sampled from a reference location (Mountain Bay) and a site within Jackfish Bay in the autumn of 2011 (10 months after mill activities ceased) and also in the autumn of 2012 (10 months after mill activities resumed). Plasma proteins were characterized using reverse-phase liquid chromatography tandem to a quadrupole-time-of-flight (LC-q-TOF) mass spectrometer and were identified by searching in peptide databases. In total, 1,367 unique proteins were identified and mapped to Gene IDs for examination by bioinformatics software to gain insight into the biological functions represented by the plasma proteins and to assess whether there were any significant changes in protein expression due to sampling location. A quantitative assay for the biomarker protein vitellogenin was also included in the LC-q-TOF analysis of fish plasmas. From each location, bioinformatics software identified networks of molecules that were related to both endocrine function and phytoestrogens (i.e., genistein and resveratrol, both of which have been detected in pulp and paper mill effluents). Plasma concentrations of vitellogenin in both male and female fish were significantly elevated in 2012 compared to 2011 ($p < 0.01$), and vitellogenin concentrations were also significantly greater in female fish sampled from the reference site compared to female fish sampled from Jackfish Bay ($p < 0.001$). Plasma protein expression profiles and vitellogenin concentrations from each year will be discussed in context with each other and also in relation to biometric endpoints, in order to understand how white sucker health is affected by pulp and paper mill activities in the Jackfish Bay Area of Concern.

Assessment of a pH-stabilized procedure to address artifactual ammonia-related toxicity for pulp and paper mill effluents (PL)

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In the pulp and paper sector, while there exists the potential for deleterious components to cause acute mortality to rainbow trout, only ammonia has been shown to have the potential to become more toxic under the regulatory test conditions. During the 96-hour regulatory test, aeration can lead to

a stripping of the carbon dioxide from the effluent, thereby causing a pH drift to occur. As the pH of the effluent increases, so too does the possibility of toxicity due to ammonia exist where it did not previously, since the potency of ammonia increases with increasing pH. At present, there are a number of pulp and paper facilities that can fail the regulatory 96-hour rainbow trout (*Oncorhynchus mykiss*) acute lethal toxicity test due to the presence of residual ammonia in the final effluent. In 2008, Environment Canada published a pH stabilization procedure (EPS 1/RM/50), which, under certain conditions, allows the toxicity related to low levels of ammonia to be addressed for wastewater effluents. FPInnovations, together with Forest Products Association of Canada (FPAC) has been working with Environment Canada to validate this pH stabilization procedure for use with pulp and paper mill effluents. The study is being conducted in three phases. Phase 1 is intended to demonstrate that the pH stabilization procedure can be successfully applied to a variety of pulping effluents, Phase 2 involves the validation of the procedure using effluents spiked with ammonia, and Phase 3 is related to the inter-laboratory validation of the procedure. This presentation will provide the results of Phases 1 and 2, which include a 60-mill survey and identification of pH drift for the major pulping processes in Canada, side-by-side comparisons for 12 effluents to demonstrate compliance with RM/50 test requirements, and ammonia-spiking experiments followed by RM/50 testing at four contract laboratories.

Reproductive effects of pulp and paper mill effluent on spawning mummichog (PO)

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Irving Pulp and Paper based in Saint John, New Brunswick, releases its mill effluent into an estuarine receiving environment. Within the context of the Environmental Effects Monitoring (EEM) program, a reproductive bioassay using the saltwater fish mummichog (*Fundulus heteroclitus*) was conducted during June-July 2012 to test the effects of final effluent on egg production and plasma sex steroid levels. After a 12-day baseline period, fish were exposed in a flow-through system to 0, 1, 5 and 10% effluent for 14 days. Each treatment contained eight aquaria with three males and three females. Egg production was not affected by treatment but plasma estradiol of females was significantly increased in fish exposed to 5 and 10% effluent. In males, the 1% effluent treatment increased testosterone levels compared with control values. Further research would be required to investigate and confirm that final effluent from the mill modulates reproductive steroid biosynthesis in exposed fish.

Changes in the isotopic signatures of white sucker (*Catostomus commersoni*) and burrowing mayflies (*Hexagenia* spp.) following the closure of an Ontario pulp mill (PO)

H. McMahon[1], T. J. Arciszewski[1], K. R. Munkittrick[1], K. A. Kidd[1]
[1]University of New Brunswick

A bleached kraft mill on the Mattagami River in Smooth Rock Falls, Ontario, was closed in 2006. Prior to closing, research was done on the impacts of pulp mill and hydroelectric activities on white sucker (*Catostomus commersoni*) and burrowing mayflies (*Hexagenia* spp.), using ratios of stable isotopes. Stable isotopes of carbon (13C/12C) and nitrogen (15N/14N) were used to determine if the benthic food web was supported by pulp mill effluent. Results of previous research (1993-1998) found an enriched 13C signature in *Hexagenia* collected directly downstream of the effluent discharge compared to those upstream of the facility; these patterns were also evident in the muscle tissue of white sucker collected at the same site. Follow-up work after the closure of the pulp mill found a residual 13C signature in the muscle and gonad tissue of the oldest white sucker collected. Concurrent work also found reduced performance in the white sucker population, suggesting a food-limited pattern of responses. The current study was initiated in 2012 to determine if a 13C signature derived from the pulp mill's effluent was remaining in the benthos (*Hexagenia* spp.) or sediment. Benthic invertebrates will also be collected in September 2013 for community analysis and comparison with previous Environmental Effects Monitoring (EEM) studies in 1995-2005. Based on the data from 2011 and 2012, we hypothesize that the isotopic signature associated with the pulp mill's effluent will not be present in the tissues of benthic invertebrates, but the community may show declines in abundance and diversity.

Distinguishing between effects of multiple effluent sources for Environmental Effects Monitoring at a pulp mill (PO)

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Treated effluent from a pulp mill is discharged into the river downstream of municipal effluent discharges. An Environmental Effects Monitoring (EEM) program was developed to distinguish between the effects of mill effluent from municipal effluent inputs within the receiving environment. An *a priori* decision matrix was developed to establish when observed "effects" (statistically significant differences between stations for a given endpoint) would be determined as "mill effects" or "municipal effluent effects". The study design was effective in determining the primary driver of effects to fish and the primary driver of effects to the benthic invertebrate community structure.

Aquaculture & Agriculture

Current status of salmon aquaculture activity in Canada with particular reference to sea lice control (PL)

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Fish farming or at-sea grow-out of Atlantic salmon is a major economic activity in Canada, providing employment in many small coastal communities in British Columbia, New Brunswick, Nova Scotia, and Newfoundland and Labrador. Growing salmon at sea comes with a number of potential negative environmental impacts, including benthic accumulation of organic material, build-up of inorganic contaminants near cage sites, and the potential for chemical therapeutants to affect indigenous non-target species. Ecto-parasites, commonly referred to as sea lice, are a problem in every jurisdiction where farming of Atlantic salmon takes place. Severe infestations of sea lice on salmon require the use of pesticides and/or drugs to limit damage and to maintain fish health. The anti-louse pesticides and drugs have the potential to affect indigenous crustacean species such as shrimp, lobsters, and crabs. The risks associated with these impacts are often specific to locations and husbandry practices. This talk will focus on potential hazards of contaminants associated with farming of Atlantic salmon in Canada and the risk associated with these hazards. In particular, the use of pesticides and drugs will be described and discussed. The presentation will serve as an introduction and an overview to set the stage for subsequent presentations of results of aquaculture-related research.

Toxicity of two pyrethroid-based anti-sea lice pesticides, AlphaMax® and Excis®, to a marine amphipod in aqueous and sediment exposures (PL)

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Pyrethroid pesticides are some of the most toxic pesticides. Marine crustaceans have been shown to be more sensitive to pyrethroids than fish, hence their desirability for use in controlling parasitic sea lice in salmon aquaculture. Deltamethrin has been shown to be the most toxic pyrethroid to crustaceans, with amphipods being one of the more sensitive species tested. Tests examining the toxicity of deltamethrin in the sea lice pesticide formulation AlphaMax® have been conducted with *Eohaustorius estuarius*, one of the amphipod species listed in Environment Canada standard test methods. However, *E. estuarius* is a Pacific species, and toxicity of AlphaMax® has not been investigated for an amphipod species from Canada's Atlantic region, where treatment of sea lice infestations in aquaculture has been occurring for many years. Toxicity data are also limited for Excis®, which contains the slightly less toxic pyrethroid cypermethrin. The present study examined the toxicity of the anti-sea

lice pesticides AlphaMax® (active ingredient [a.i.] deltamethrin) and Excis® (a.i. cypermethrin) to amphipods collected from tide pools along Passamaquoddy Bay, in the Bay of Fundy region of New Brunswick. The collected species was identified as *Echinogammarus finmarchicas*, an amphi-Atlantic, shallow-water gammaridean amphipod considered a dominant species of tide pools along bedrock surf coasts and salt marsh pools. Amphipods were exposed to the two different pesticides in acute (1- and 24-hour) water-only exposures and 10-day sediment tests. Our goal was to fill in data gaps with respect to the comparative toxicity of these anti-sea lice pesticides in both aqueous and sediment exposures and the sensitivity of amphipod species.

Susceptibility of benthic invertebrates to agricultural effects in New Brunswick's small streams (PL)

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Agriculture is large-scale landscape disturbance known to affect stream water quality, habitat, and biological communities. In 2010 and 2011, invertebrate communities in 14 streams along a gradient of agricultural land use were studied in one of New Brunswick's high-intensity potato production regions, Grand Falls. Benthic invertebrate samples were collected by U-net at the end of August from riffle areas and the effects of agriculture were assessed on community indicators of diversity, sensitivity, habitat use, feeding strategy, and productivity. Analysis of landscape and site characteristics showed that study sites were differentiated most strongly by the percentage agriculture in their catchment, but that stream catchment area also influenced sites. Assessment of the invertebrate data showed that percentage agriculture and catchment area had opposing effects. Benthic invertebrate diversity, sensitivity, and productivity indicators tended to be negatively affected by agriculture but positively affected by catchment area. Habitat use and feeding strategy of the invertebrate taxa showed a shift in the community from clingers, collector filters, and predators in streams with lower-percentage agriculture to burrowers, swimmers, and collector gatherers from sites with higher-percentage agriculture. In contrast, this same shift in habitat and feeding traits was observed, to a lesser extent, with decreasing catchment area. These results indicate that the negative effects of agriculture appear to be most apparent in smaller streams.

Photodegradation of pharmaceuticals in aquatic environments: Defining our current understanding and identifying knowledge gaps (PL)

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This presentation summarizes an ongoing critical assessment of the state and quality of knowledge around the aquatic photochemistry of human-, veterinary- and agricultural-use pharmaceuticals in laboratory experiments and field observations. We use a weight of evidence

approach to identify knowledge gaps in the literature regarding the photolytic fate of pharmaceuticals in surface waters. A special sub-focus is on characterizing the ability of current laboratory studies to predict fate under natural field conditions. A standardized scoring rubric was used to assess the quality of the methodology and experimental design of relevant laboratory and field studies. The rubric provides a consistent method for assessing the merit of a scientific study, and will provide a benchmark for the design of future photolytic fate studies. The scores then allow for a simple, easily interpreted two-dimensional plot (x-axis = laboratory studies, y-axis = field studies) to assist in identifying significant knowledge gaps in our understanding of photolysis by pharmaceutical class, as well as the quality of the data generated to date. This permits recommendations as to where future research needs to be directed in order to improve our understanding of pharmaceutical fate and exposure in surface waters for human and ecological risk assessments.

Zinc and calcium act synergistically to impair rainbow trout mitochondrial respiration and membrane potential (PL)

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To understand the interactions that typically occur between co-occurring stressors in the aquatic environment, the present study investigated the effects of zinc (Zn) and calcium (Ca) on mitochondrial bioenergetics. Zn, an essential trace metal, is a priority aquatic pollutant whose toxicity in fish is mitigated by Ca due to competition for uptake pathways on the gill surface. To the best of our knowledge, nothing is known about post-absorption Zn-Ca interactions in fish at lower levels of biological organization, including mitochondria. Mitochondria are the powerhouses of the cell, generating 95% of an organism's energy. Co-occurring stressors may act interactively on these organelles with implications for apical endpoints of toxicity, including survival, growth, and reproduction. The present study therefore investigated the effect of Zn and Ca, singly and in combination, on mitochondrial bioenergetics *in vitro* with a view to identifying the mechanisms and sites of cellular energy impairment caused by the two stressors. Mitochondria were isolated from rainbow trout (498 ± 105 g) livers and exposed to 0-100 μM Zn and Ca singly and in equimolar combinations. The effects on malate-glutamate-driven oxidative phosphorylation (OXPHOS) and mitochondrial complex I (mtCI) enzyme activity were then assessed. As well, real-time flow cytometry-based fluorescence detection of the membrane potential-sensitive cationic lipophilic dye, JC-1, was conducted to reveal the effect of these stressors on mitochondrial inner membrane electrochemical gradient. The results show that, although both Zn and Ca impaired OXPHOS, Ca was much less potent than Zn, based on a six-fold higher state 3 mitochondrial respiration and mtCI activity Zn EC_{50} values. Interestingly, and contrary to our hypothesis that Ca would be protective against Zn-induced mitochondrial toxicity, the two elements acted synergistically to impair OXPHOS. Specifically, the state 3 mitochondrial respiration EC_{50} was 2.5 and 16.5 times lower in the combined relative to the Zn and Ca alone exposures, respectively. Despite imposing a lower inhibitory effect on state 3 respiration, real-time flow cytometry revealed a greater disruptive effect of Ca on mitochondrial membrane potential than Zn. These findings indicate that Ca and Zn do not exhibit antagonistic interactions at the

mitochondrial level and that the greater mitochondrial toxicity of Zn relative to Ca is not explained by membrane potential disruption.

Analysis of gene expression in *Homarus americanus* larvae exposed to sublethal concentrations of endosulfan during metamorphosis (PO)

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Agricultural pesticide runoff has been suspected as the cause of numerous fish kills in rivers throughout Prince Edward Island, but the impact on the surrounding marine environment is unknown. Endosulfan, an organochlorine pesticide, is a potent neurotoxin and moult inhibitor used to combat the Colorado potato beetle; however, it poses a threat to non-target organisms, including the American lobster (*Homarus americanus*). Metamorphosis is a critical stage of development and the effects of contaminant exposure during this time are largely unknown in lobster. A 14-day endosulfan exposure was performed to identify the effects on survival, development, and gene expression in lobster larvae during metamorphosis. A custom-made *H. americanus* microarray was used for monitoring the changes in expression of 14,592 genes at the termination of the exposure. A total of 707 genes were identified as having a fold change > 1.5 and being significant at $p < 0.05$ using one-way ANOVA. Annotated genes of interest were involved in many processes, including development, metabolism, immunity and oxidative stress response and gene regulation. Nine genes of interest (histone H1, farnesoic acid o-methyltransferase, cuticle protein, glutathione-S-transferase, thioredoxin, NADH dehydrogenase, ecdysone nuclear receptor Fushi tarazu F1 [FTZ-F1], ferritin, and ecdysone inducible protein E75 [EIP-E75]) were selected for RT-qPCR validation of the microarray results. The RT-qPCR method was more sensitive than the microarray, yet detected similar expression patterns. The two highest endosulfan concentrations resulted in increased mortalities, developmental delays in reaching metamorphosis, and significant changes in gene expression. This research provides a foundation for using microarray gene expression profiles as screening tools for exploring the impact of environmental contaminants on lobster.

The effects of freshwater cage aquaculture on sediment quality in the Great Lakes (PO)

N. Diep[1], D. Boyd[1], R. Sein[1], L. Vandermeer[1], M. Tybinkowski[1], T. Watson-Leung[1]
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In Ontario, commercial-scale freshwater cage aquaculture operations are located in the upper Great Lakes, primarily Lake Huron/Georgian Bay. They discharge large quantities of nutrients and oxygen-consuming materials (i.e., fish feces) directly into the natural environment, and this can lead to significant depletion of dissolved oxygen and other eutrophication effects. We found water quality effects to range from negligible effects at exposed, well-flushed sites to eutrophication effects at

sheltered, sensitive sites. Three waterbody categories were identified according to their site-specific characteristics and potential sensitivity to discharges from cage aquaculture operations. Types 1 and 2 sites are embayments that are lake-like with limited connectivity to the open waters of the Great Lakes. These categories are more sensitive to waste discharges and more likely to experience eutrophication effects because of their limited assimilative capacity (e.g., locally enclosed with little/no hypolimnetic exchange). Hypolimnetic anoxia, seasonal phosphorus enrichment (> 10 $ug \cdot L^{-1}$), and occurrence of algal blooms were observed in these systems. Type 3 sites are highly exposed sites with good bottom water exchange and did not exhibit eutrophication effects. Our data demonstrate that appropriate siting and operational practices that minimize waste discharges are an integral component of reducing the risk of water quality impairment. We provide an overview of key research highlights from our water quality and real-time continuous *in situ* monitoring studies.

The effects of freshwater cage aquaculture on water quality in the Great Lakes (PO)

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In Ontario, extensive information on sediment quality was collected by the Ontario Ministry of Environment to assess the environmental conditions in and around commercial-scale freshwater cage aquaculture operations in the Great Lakes. Commercial-scale cage aquaculture operations discharge large quantities of nutrient-rich, high biological oxygen demand (BOD) solid waste, primarily feces and some waste feed, to the lakebed. These waste deposits were found to alter sediment conditions in the immediate vicinity of the cages. Local sediment-quality effects ranged from enriched sediment conditions with high densities of pollution-tolerant macroinvertebrates to areas where sediment chemistry, exceeding the provincial sediment quality guidelines "severe effect" levels (PSQG-SELs), exhibited sediment toxicity and were devoid of macroinvertebrates. High accumulations of these waste deposits resulted in elevated pore water nutrient concentrations, low redox conditions, and the potential for un-ionized ammonia and hydrogen sulphide production, which can lead to a habitat toxic to most aquatic organisms. We used sediment bioassays to quantify the response of a pollution-tolerant deposit-feeder, *Lumbriculus variegatus*, to various fecal accumulation rates. At low accumulation rates, we observed biostimulation and waste assimilation occurring through high growth and reproduction. At high accumulation rates, growth rates were inhibited. A depositional model was used to predict the sizes and shapes of the waste depositional footprints for operations of varying cage configurations, fecal settling velocities, and site characteristics. We found options that result in a larger waste footprint resulted in lower accumulation rates, increasing the potential for waste assimilation. Our data demonstrate that appropriate siting and operational configurations and practices that minimize waste accumulation rates are integral to minimizing sediment-quality effects and will result in enriched, non-toxic sediment conditions with increased potential for waste assimilation. We provide an overview of the key research highlights from our sediment chemistry, benthic invertebrate community, and sediment toxicity bioassay assessments.

Transport of three veterinary antimicrobials from feedlot pens via simulated rainfall runoff (PO)

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Veterinary antimicrobials are used in animal production therapeutically, to treat diseases, and sub-therapeutically to prevent diseases and promote growth. There is a growing concern that environmental contamination by these chemicals may result in widespread bacterial resistance to antimicrobials used in human medicine. The majority of administered antimicrobials (up to 80% or more) are excreted in the feces or urine either as parent compounds or their metabolites. These compounds are introduced into the environment mainly through manure applied as fertilizer to agricultural fields or through leaching or runoff from manure storage locations (stockpiles, windrows, lagoons). Veterinary antimicrobials have been detected in manure, manure-treated soils, and surface and ground water resources near large-scale cattle feeding operations. Runoff and leaching after major precipitation events appear to be major transport pathways by which these veterinary antimicrobials eventually contaminate surface and ground water. A study was conducted in a research feedlot situated at the Agriculture and Agri-Food Canada Research Centre at Lethbridge, Alberta, in spring 2011 to investigate the transport in surface runoff of three antimicrobials commonly used in beef cattle (*Bos taurus*) production. Beef cattle were administered the following antimicrobial treatments: (1) 44 mg of chlortetracycline per kg feed (dry-weight), (2) 44 mg of chlortetracycline plus 44 mg sulfamethazine per kg feed, (3) 11 mg of tylosin per kg feed and (4) no antimicrobials (control). Three pens per treatment were selected, and concentrations of the three antimicrobials were measured in simulated rainfall runoff from two locations (bedding and floor areas) randomly selected in each feedlot pen. Manure and runoff sample extracts were analyzed for chlortetracycline, sulfamethazine, and tylosin using LC-MS-MS. The residue data will be used to investigate the transport of the three antimicrobials in runoff and potentially generate information to support the inclusion of retention ponds in feedlots to capture runoff from feedlot pens, thereby minimizing the risk of contamination of surface and groundwater resources.

Feeding response in marine copepods as a measure of sublethal toxicity of four anti-sea lice pesticides (PO)

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Copepods are the most abundant organisms in the mesozooplankton and an important component of marine and estuarine food webs. Zooplankton may potentially be exposed to anti-sea lice pesticides released from aquaculture sites, particularly if entrained in an effluent plume. Laboratory bioassays were conducted to determine the acute toxicity of four sea lice pesticides to copepods routinely collected from Passamaquoddy Bay, in the Bay of Fundy region of New Brunswick. The

pesticides investigated were AlphaMax® (active ingredient [a.i.] deltamethrin), Excis® (a.i. cypermethrin), Interox®Paramove 50 (a.i. hydrogen peroxide) and Salmosan® (a.i. azamethiphos). Copepods were exposed to a pesticide for 1 hour followed by 5 hours of recovery in clean water, and endpoints were assessed using staining techniques. Feeding was assessed by providing carmine particles to copepods for the final 2 hours and lethality was assessed separately with the vital stain Neutral Red. In tests with AlphaMax®, Excis®, and Interox®Paramove 50, organisms exposed to high test concentrations were typically immobilized (sunk to the bottom of test beakers) within the 1-hour exposure. At the end of the 5-hour recovery, all organisms were stained with Neutral Red, including those immobilized at high test concentrations, indicating they were still alive. As a result, a concentration-response typically was not observed with the vital stain, and LC_{50} values could not be determined. However, feeding behaviour of copepods was affected in tests with AlphaMax®, Excis® and Interox®Paramove 50, as the proportion of copepods with carmine particles in their digestive tracts decreased with increasing exposure concentration. The estimated EC_{50}s for feeding based on measured concentrations of active ingredient ranged from 0.017-0.081 $\mu g \cdot L^{-1}$ for AlphaMax®, to 0.098-0.55 $\mu g \cdot L^{-1}$ for Excis®, and to 2600-8300 $\mu g \cdot L^{-1}$ for Interox®Paramove 50. These EC_{50}s represent approximately 25 to 117-fold, 9 to 51-fold, and 145 to 460-fold dilutions of the aquaculture treatment concentrations for these pesticides, respectively. Copepods exposed to Salmosan® were not immobilized even at 5-times the aquaculture treatment concentration, and a consistent concentration-response of feeding was not observed in repeated bioassays negating calculation of an EC_{50} value. In summary, for three of the four sea lice pesticides studied, acute exposure affected feeding and mobility of zooplankton in laboratory tests at environmentally realistic concentrations.

Ecological Risk Assessment

Using non-linear models to predict the chronic toxicity of cobalt to *Hyalella azteca* under different water chemistry conditions (PL)

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The federal Chemicals Management Plan is examining cobalt (Co)-containing substances of interest. These substances are released into the environment from sectors such as alloy manufacturing, smelting and refining, and petroleum refineries. Site-specific water chemistry is an important factor in predicting whether a given concentration of a metal will be toxic. Dissolved organic carbon (DOC) concentrations, pH and alkalinity levels are some of the factors that may impact toxicity by altering the bioavailability of the metal through competition or complexation with other ligands or ions. Growth, mortality, and bioaccumulation endpoints were investigated after 28 days in the amphipod *Hyalella azteca* following exposure to a concentration series of cobalt chloride at three different levels of pH, DOC, or alkalinity in soft water. The results indicate negative effects on both growth and mortality when *H. azteca* is exposed to increasing Co concentrations. Lethal and effective concentrations were determined using non-linear mortality and growth models. The least- and most-sensitive endpoints vary between the different water chemistry conditions; however, the growth endpoint is generally more variable. It was found that increased pH decreases mortality, possibly due to the reduction in the Co free ion. Increased DOC and decreased alkalinity resulted in a non-significant decrease in mortality. The data were also compared to previous 7-day exposures in soft water and 28-day exposures in moderately hard water.

Lethal and sublethal toxicity of nitrates and phosphates in the endemic fish *Skiffia multipunctata* (PL)

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Mexican freshwater fish fauna are highly diverse. The Goodeinae family, endemic to the Mexican Central Plateau, is considered among the representative groups. These fish are viviparous. It is estimated that more than half of the group's species are endangered due to the deterioration and high levels of pollution in the water bodies they inhabit. This paper determined the effect on the endemic fish *Skiffia multipunctata* (Pellegrin, 1901) of lethal and sublethal concentrations of nitrates and phosphates to establish their respective tolerance intervals. Concentrations of 0.0, 0.016, 0.028, 0.049, 0.085 and 0.15 $mg \cdot L^{-1}$ of nitrites and 0.0, 1.62, 3.08, 5.86, 8.79, 13.18 and 19.77 $mg \cdot L^{-1}$ of phosphates, with 3 repetitions each (n=30), were used to obtain the $LC_{50,}$ following Organisation for Economic Co-operation and Development (OECD) procedures. During the exposure of the fish to both toxic

compounds, erratic swimming, collisions with the tank, presence of wounds, and mortality were observed. The calculated LC_{50}s were 0.064 $mg\cdot L^{-1}$ for nitrates and 13.18 $mg\cdot L^{-1}$ for phosphates at 24 and 96 hours, respectively. The LC_{50}s of both toxics that caused severe alterations at gill level (hyperplasia, hypertrophy, and lamellar fusion) are lower concentrations than those marked as limits in the Official Mexican Norm (NOM-001-SEMARNAT-1996) in order to conserve the wild aquatic fauna. Period of exposure and concentration were decisive in the effect.

Ecological screening risk assessment of mitotane, a pharmaceutical active ingredient, conducted as part of Canada's Chemicals Management Plan (PL)

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Mitotane, or o,p'-DDD, is a chemical that may be formed in the environment by degradation of o,p'-DDT. It is also the active ingredient in a drug used to treat cancer of the adrenal gland. This chemical has been the subject of a recent screening risk assessment conducted by Health Canada and Environment Canada under the *Canadian Environmental Protection Act, 1999* (CEPA 1999) as part of the Chemicals Management Plan. Based on mitotane's chemotherapeutic use, a small amount of this substance may be excreted in feces and released to wastewater systems. While limited quantities of mitotane are used in Canada, a relatively large proportion of that amount may be released at a small number of sites, such that concentrations of mitotane could be sufficiently high in the receiving aquatic environment to be of concern, given its high toxicity to aquatic organisms. A risk quotient analysis was performed by comparing the estimated exposure of aquatic organisms with concentrations expected to cause harm. The results of this risk quotient analysis, along with other considerations such as the substance's toxicological mode of action and metabolic pathway, and its high persistence and bioaccumulation potential, led to the proposed conclusion that mitotane may enter the environment under conditions that may have a harmful effect on the environment. The multiple line-of-evidence approach used in screening assessments under CEPA 1999 and the tools used to estimate the exposure in the Canadian environment will be discussed.

Canada's chloride guideline: Too high to protect a native daphniid species in soft water lakes (PL)

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Water quality regulations for freshwater aquatic life have been based mainly on the effects of contaminants on a few species. Daphniids are the most widely used cladoceran species for water quality tests and have been used to set regulatory guidelines. Researchers can choose from among multiple media for these acute and chronic toxicity tests, but most are hard water media. The objective of our study was to determine the sodium chloride (NaCl) and chloride (Cl) LC_{50} values in soft water for a native

daphniid species living in soft water lakes in Ontario. We ran 14-day chronic, with 48-hour renewal bioassays, in Conviron E7/2 growth chambers at 20°C and with a 16:8 hour light:dark cycle. We used <24-hour neonates of a local isolate of *Daphnia pulicaria*, a common inhabitant of Ontario lakes. Bioassays were performed in a chemically defined, soft water medium (the FLAMES medium), which has been designed to reflect the chemistry of non-acidic, soft water Canadian Shield lakes that support a diverse and stable daphniid assemblage. The daphniids were fed *Pseudokircheriella subcapitata* and *Scenedesmus obliquus* at 1 mg $C \cdot L^{-1} \cdot day^{-1}$. The bioassay had excellent control survival, monotonic response of survival in the treatments, and stable endpoints after ten days. The 14-day Cl LC_{50} in this soft water medium for *Daphnia pulicaria* was 74.01 $mg \cdot L^{-1}$, about half the current chronic Canadian water quality guideline for the long-term protection of aquatic life. Clearly, *D. pulicaria* isolated from a native lake is very sensitive to chloride in soft water. Sodium and chloride levels are increasing in many Ontario lakes, and lakes are getting softer as calcium (Ca) levels decline. Because there are many soft water lakes in Ontario, it is important to consider that soft water lakes may need specific chloride guidelines for the protection of their biota.

Federal environmental quality guidelines for chlorinated alkanes, vanadium, bisphenol A (BPA), tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD) (PO)

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Federal Environmental Quality Guidelines (FEQGs) are developed under the *Canadian Environmental Protection Act, 1999* (CEPA 1999) to support federal environmental quality monitoring, risk assessment and risk management activities as identified in the Chemicals Management Plan and other federal initiatives. FEQGs provide benchmarks for the quality of the ambient environment. Where the FEQG for a given environmental medium is met, there is low likelihood of adverse effects on protected use (for example, a water quality guideline protects aquatic organisms, a wildlife diet guideline protects wildlife species that consume aquatic biota, and a sediment environmental quality guideline protects sediment-dwelling organisms). FEQGs are based on the toxicological effects or hazards of specific substances or groups of substances and do not take into account analytical capability or socio-economic factors. The use of FEQGs is voluntary unless prescribed in permits or other regulatory tools. FEQGs can aid in preventing pollution by providing targets for acceptable environmental quality, help evaluate the significance of concentrations of chemical substances currently found in the environment (monitoring of water, sediment, and biological tissue) and serve as performance measures of the success of risk management activities. The first set of FEQGs, which was published in February 2013 for alcohol ethoxylates, cobalt, hydrazine, and polybrominated diphenyl ethers (PBDEs), is available online. Currently, FEQGs for chlorinated alkanes, vanadium, bisphenol A (BPA), tetrabromobisphenol A (BPA) and hexabromocyclododecane (HBCD) are being developed at Environment Canada. The draft FEQG values and the supporting information for these substances will be presented.

Review of the toxicity, exposure, and environmental fate of polybrominated diphenyl ethers (PBDEs) in the terrestrial environment (PO)

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Brominated flame retardants are a ubiquitous group of chemical compounds typically used in a wide range of residential and household applications to slow down the rate of combustion. Polybrominated diphenyl ethers (PBDEs) are a well-established class of persistent, bioaccumulative brominated flame retardant additives that exhibit adverse effects on all environmental compartments. With a total of 209 known PBDE congeners, they can be further simplified into the following three classifications based on increasing degree of molecular mass, size, and bromination: pentaBDE, octaBDE and decaBDE. These toxic organic compounds were first introduced into the manufacturing process in 1965 and are now widespread in finished products such as electronics, electrical devices and equipment, paints, textiles, plastics, and polyurethane foams. As consumer demands increase, the concentrations of PBDEs will continue to rise. At the end of the product life cycle, the disposal of PBDE-containing waste represents a major area of concern, resulting in increased point source emissions found entering the terrestrial environment. Soil and sediments represent the most favoured environmental reservoirs for PBDEs due to the relatively low water solubility and high partitioning coefficients. The common modes of entry into the soil environment include wet or dry atmospheric deposition and adsorption from the atmosphere to vegetation, plants, and organic matter. Overall, due to the complex nature and chemical reactivity of PBDEs, the adverse effects within the terrestrial environment will be further investigated through the use of an integrated ecological risk assessment framework.

Assessment of the disruption of steroidogenesis in three North American fish species by use of an *in vitro* gonadal explant assay (PO)

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There is concern regarding exposure of aquatic organisms to chemicals that interfere with the endocrine system. One critical mechanism of endocrine disruption is impairment of steroidogenesis, which can lead to altered hormone levels, altered or delayed sexual development and, ultimately, reproductive failure. There is also a need to address the large number of animals required by current toxicity testing approaches, particularly when working with species native to environments of concern. The aim of this study was to develop an *in vitro* gonadal explant assay enabling the assessment of endocrine-disrupting chemicals on sex-steroid production in wild fish species native to North America. Northern pike (*Esox lucius*), walleye (*Sander vitreus*) and white sucker (*Catostomus commeroni*) were sampled from Lake Diefenbaker, Saskatchewan, Canada at pre-spawn and multiple post-spawn time-points. Blood was taken and plasma was analyzed for 11-ketotestosterone (11-KT) and estradiol (E2). Gonads were excised and exposed for 24 hours to a model inducer (forskolin) and inhibitor (prochloraz) of steroidogenesis. Hormone concentrations in plasma and media were quantified using ELISA. The

seasonal profile of plasma hormones had a trend similar to the seasonal profile of basal hormone production in gonadal tissue exposed to the solvent control. Tissues exposed to forskolin showed a concentration-dependent increase in 11-KT and E2. Exposure to prochloraz resulted in a decrease of 11-KT and E2. These results illustrate that the gonadal tissue is undergoing steroidogenesis in an *in vitro* setting that is reflecting reproductive seasonality and is also responsive to chemical exposure in a concentration-dependent manner. White sucker were found to be the most responsive species, with the seasonal time-point of greatest sensitivity differing between sexes. When exposed to 3 μM forskolin, white sucker males and females produced up to 15-fold greater 11-KT and 15-fold greater E2, respectively, when compared to the solvent controls. In conclusion, seasonality of reproductive function represented a critical factor that needs to be considered when using the here-established *in vitro* explant assay to assess responses of native species to disruptors of steroidogenesis. Further characterization of molecular and biochemical endpoints in the gonads and liver, and comparisons to *in vivo* effects, are ongoing in an effort to validate these *in vitro* methods as a predictive ecotoxicological tool.

Metal uptake by aquatic plants in a remedial and restoration application (PO)

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The application of phytoremediation technologies to reduce concentrations of contaminants from terrestrial and aquatic environments has steadily grown in North America as an effective and low-cost alternative to traditional remedial methods. In general, phytoremediation uses plants' inherent abilities of uptake, degradation, or transposition to move contaminants from the surrounding media. In particular, wetlands complexes have been used to successfully treat metals, hydrocarbons, wastewater, and industrial chemicals. As an alternative to a financially prohibitive cleanup and/or excavation, an engineered wetland was constructed as a means to remove metals from a contaminated site in northeastern Nova Scotia. The complex consisted of three interconnected ponds separated by highwater swales, with each pond containing assemblages of carefully selected wetland plants. This allowed for a low cost, proactive, ongoing water treatment prior to discharge into a nearby watercourse. Analytical results were used to verify the effectiveness of this approach and emphasize the importance of controlling factors, such as differing uptake ability by various plants, spatial distribution, and preferred habitat. Focus of presentation will be on metals of concern such as aluminum, iron, manganese and zinc. Comments will be provided on naturalizing the wetland over time, as well as the general ability of the wetland as a whole to improve water quality.

Acute and chronic toxicity of aqueous vanadium to model and field-collected planktonic and benthic invertebrates (PO)

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Vanadium (V) is a widely distributed trace metal occurring abundantly as organic complexes in many crude oils, including bitumen from the Alberta oil sands. As a result of bitumen upgrading, V is highly concentrated into the oil sands by-product coke. Previous research has demonstrated the mobilization of V from coke into solution to be largely favored under alkaline conditions. Such elevated concentrations of V could pose ecological risks to surrounding freshwater systems within northern Alberta. However, given that the current understanding of V toxicity to aquatic organisms is limited, risks associated with such concentrations to aquatic systems are difficult to estimate. Therefore, the goal of these studies was to investigate the acute and chronic toxicity of V to planktonic and benthic invertebrates, comparing the responses of two standard test species, *Daphnia pulex* and *Chironomus dilutus*, to those of comparable species collected from northern Alberta. Test measurements included V in overlying waters, where bioavailable monomeric vanadate oxyanions are predicted to be the dominant species. Acute toxicity (48-hour LC_{50}) for *D. pulex* exposed to V was determined to be 2.23 $mg{\cdot}L^{-1}$. During a 21-day chronic exposure, lethality was reduced to 0.46 $mg{\cdot}L^{-1}$. Significant effects (81.9% reduction) in reproduction were observed at concentrations $\geq$ 0.63 $mg{\cdot}L^{-1}$, with an EC_{50} for reproductive impairment of 0.45 $mg{\cdot}L^{-1}$. Toxicity endpoints for *C. dilutus* included an acute 96-hour LC_{50} of 49.2 $mg{\cdot}L^{-1}$. Growth at 14 days and adult emergence rates (25.9% and 31.6%, respectively) were both significantly reduced at nominal V concentrations $\geq$ 16.2 $mg{\cdot}L^{-1}$. Analogous toxicity tests are currently being conducted with field-collected Cladocera and Chironomidae. These studies not only aim to improve our understanding of the effects of V on freshwater invertebrates, but to determine if differences in sensitivity exist between field-relevant species and commonly used laboratory test species, and how these could influence the development of site-specific water quality guidelines for V.

Innovative Tools: The Cool Stuff

Targeted transcriptomics of environmental toxicants across multiple taxa (PL)

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Investigating the link between environmental toxicants and the community structure of aquatic bioindicator species is part of an ongoing effort to monitor long-term trends, track the progress of existing clean-up efforts, and sustain natural aquatic habitats throughout the world. Despite this effort, a gap in understanding exists between changes to community structure, toxicological response, and the functional (i.e., gene expression) consequences of environmental pollution. Chemical monitoring efforts are limited by timing as well as analytical costs and technology in their ability to identify sites of concern. With the decreasing cost of genomics and transcriptomics, these approaches could provide a time-sensitive "early warning system" to screen for sites with potential biological impact, thereby focusing resources on sites of known concern. Targeted transcriptomics is a novel method that uses functional gene expression, in this case, of selected genes. Differential patterns of gene expression of these targeted genes may provide an indication of the functional response to pollutant levels at a molecular level. The aim of this in-lab ecotoxicogenomic study is to identify the relationship between targeted functional gene expression and physiology of aquatic organisms exposed to PCB-52, a common congener found in sediments of several freshwater systems in Ontario. Notable endocrine and reproductive disruption, neurotoxic, immunotoxic and carcinogenic effects have been observed in wildlife across the globe. PCB-interacting genes have been identified and will be used for developing probe sets for the targeted transcriptomics analysis using the Illumina MiSeq platform. For this research, we will initially focus on *Hexagenia* spp., a burrowing mayfly native to Ontario. *Hexagenia* spp. is an important bioindicator species that ingests sediment and is known to accumulate PCBs in its tissue, thereby acting as a source of PCBs to the terrestrial food web upon emergence. The ultimate goal of the development of targeted transcriptomics technology is to develop a method for identifying multi-taxa xenobiotic-interacting genes and determine possible relationships between the targeted functional gene expression with physiology and, ultimately, toxicology of aquatic organisms exposed to PCBs. Future work will involve applying this technique to PCB-contaminated aquatic communities for early detection of toxicity in nature.

Hepatic gene expression profiles in the female fathead minnow (*Pimephales promelas)* liver following acute and sub-chronic exposures to phenanthrene (PL)

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Phenanthrene (PHEN) is a polycyclic aromatic hydrocarbon (PAH) that is associated with coal, oil deposits, and municipal and industrial wastewaters. PAHs have been shown to induce oxidative stress in teleost fish; however, studies also suggest that PHEN can disrupt reproductive endpoints. Female fathead minnow (FHM; *Pimephales promelas*) were exposed to PHEN in both acute and sub-chronic bioassays. Adult reproductive FHM were first acutely exposed to measured concentrations of 0, 29.8, 389.4 and 942.5 $\mu g \cdot L^{-1}$ for 24, 48 and 72 hours, where the low and medium concentrations represent environmentally relevant levels. Adult FHM minnow were also sub-chronically exposed to an averaged measured concentration of 201.8 $\mu g \cdot L^{-1}$ over approximately 7 weeks (47 days). FHM acutely exposed to PHEN did not show impacts on 17β-estradiol levels, condition factor and organ indices when compared to control fish. However, microarray analysis at 48 hours showed that there were 1235, 907 and 2568 differentially regulated transcripts with 29.75, 389.4 and 942.5 $\mu g \cdot L^{-1}$ PHEN, respectively. Of these, 228 transcripts were differentially expressed at all three concentrations. Gene set enrichment analysis revealed ribosomal synthesis and activity, androgenic biosynthesis pathways and chemokine activity were impacted by PHEN at each concentration. FHM females sub-chronically exposed to 201.8 $\mu g \cdot L^{-1}$ PHEN for 47 days displayed altered swimming behaviour, decreased condition factor and gonadosomatic index when compared to unexposed fish. Gene expression patterns in the liver will be compared to those at 47 days. These data improve our understanding regarding the modes of action for acute and sub-chronic exposures to PHEN both prior to and after higher-level tissue effects are detected. Due to the abundance of PAHs in the environment from metal mining and oil extraction processes, understanding the potential of low molecular weight PAHs to disrupt endocrine processes will assist in improved characterization of the risks associated with acute and longer-term exposures.

Laser ablation inductively coupled mass spectrometry (LA-ICPMS) tool enhanced for use in non-lethal temporal characterization of metal exposure in wild mammals (PL)

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We enhanced an existing technique, laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS), to function as a non-lethal tool in the temporal characterization of metal exposure in wild mammals. Mercury (Hg) and grizzly bears (*Ursus arctos horribilis*) were chosen as the metal and wildlife species, respectively, for initial stages in method development, as salmon-derived Hg was expected to provide strong temporal signals in coastal grizzly bears of British Columbia. Our method development included three major steps. Step 1 was optimization of laser parameters for grizzly bear hair, with 2000 μm line scans along the hair at a speed of 50 $\mu m \cdot s^{-1}$ (spot size = 30 μm). The reference

material DOLT2, dogfish liver powder pressed into a pellet, and sulphur were selected as our external and internal standards, respectively. Step 2 was quality assurance / quality control analyses, with hair analyzed using both LA-ICPMS and standard ICPMS. Hg concentrations obtained with these two methods were highly correlated ($R^2 = 0.96$; $p = 0.008$). In addition, the reproducibility of the technique was assessed by analyzing several hairs from the same bear and by running the hair under the laser from tip to root and then root to tip. Cross-correlation analyses revealed high correlations amongst replicates. Step 3 was validation, with LA-ICPMS analyses performed on the hair of five captive grizzly bears fed various amount of cutthroat trout for a period of 33 days. Hg patterns along the hair revealed strong Hg signals coinciding with fish consumption, and a significant correlation between Hg in hair and Hg intake was evident (Hg hair [$\mu g \cdot g^{-1} \cdot day^{-1}$] = 0.030 x Hg intake [$\mu g \cdot kg^{-1}$ bear$\cdot day^{-1}$] = 0.0049; $R^2 = 0.97$; $p < 0.001$). This is the first study evaluating temporal metal exposure and accumulation in wildlife using LA-ICPMS. The high temporal resolution in metal exposure obtained through LA-ICPMS in hair will be a great asset in future wildlife health monitoring programs.

Post-industrial intertidal biodiversity and habitat restoration in Howe Sound, British Columbia (PL)

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Studies conducted in 1990-1993, 2004, 2012, and 2013 have shown that biodiversity of intertidal species in the mid- to low-tide zone decreased significantly as exposure to pulp mill and mine effluent increased along pollution gradients in Howe Sound, British Columbia. During the earliest Howe Sound surveys, two pulp mills (Woodfibre and Port Mellon) were operational and, since the 1990s, successful pollution abatement was undertaken to decrease the toxicity of marine effluent. This research describes the recovery of intertidal biodiversity at the most highly impacted sites, Darrell Bay (proximal to the decommissioned Woodfibre pulp mill), Port Mellon (proximal to the operational Howe Sound Pulp and Paper Mill) and Britannia Beach (drainage site for decommissioned Britannia Mine). At Darrell Bay, intertidal biodiversity increased from six species in the 1990s to 12 species in 2004 (post-pollution abatement) and to 16 species in 2013 (post-mill closure). Experiments investigated whether recolonization and recovery are accelerated at the Darrell Bay site by providing additional rocky intertidal habitat through rock transplantation to the mid-low tide zone. Recruitment of five species to naïve rock was observed by six weeks and, by 12 months, 10 species were present. However, by 14 months, a reduction in species richness was observed (7 species remaining), likely a result of the physical disruption of this site by an influx of wood fibres onto the beach that formed a mat on two of the four replicate rock transplants. Although both Britannia Beach and Port Mellon rocky intertidal sites exhibit significantly lower biodiversity compared to reference sites, the species richness at both of these impacted sites has increased since the earliest 1990-1993 surveys and by ≥ 2-fold between the 2004 and 2013 surveys. These studies indicate increases in species diversity following pollution reduction; however, further time and potentially remediation of historical contamination with habitat augmentation are likely necessary for recolonization to the level of reference sites.

Biomonitoring 2.0: Generating and harnessing data on an epic scale for ecosystem assessment (PL)

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Two late-20th century technologies, earth observation systems and the Internet, have massively increased available data on the physical environment, fostering a plethora of journal papers on the state of our natural ecosystems. However, most stumble at the last data hurdle, on the realization that there is a fatal scale-mismatch between physical and chemical observations, which can be used to generate hypotheses about ecosystem status and observations on biota, which must form the basis of any meaningful assessment. Ecosystem-scale biomonitoring is currently mired in old-tech thinking, having failed to progress from its comfort zone of site-level assessments of general impairment towards the incorporation of diagnosis of causality. Statistical methods for the extraction of stressor-specific patterns from noisy environmental data exist, yet their use is restricted not just by limited quantity, but also by the patchy spatial coverage and inherently gnarly nature of biological observations. Recent developments in genomics, such as next-generation sequencing, are transforming other areas of the life sciences, but their application in ecological assessment is limited by cost and infrastructure accessibility, and also by the "shock of the new" effect posed by their transformative nature. This is all about to change, however, and in this presentation, we anticipate the advent of cheap and freely-available high-throughput sequencing, including the development of DNA-based taxonomy, illustrating their potential to transform the quantity, scale, and accuracy of biological observation through reference to specific examples from terrestrial and freshwater ecosystems.

The chronic effects of hydroxypropyl-β-cyclodextrin on the reproduction of the American flagfish (*Jordanella floridae*) over one complete life cycle (PO)

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The impact of pharmaceuticals and personal care products (PPCPs) on aquatic organisms has become an important issue for aquatic toxicologists. Many PPCPs have been shown to cause non-target effects on aquatic biota within detected environmental ranges. Of particular interest are the potential toxicological effects of hydroxylpropyl-β-cyclodextrin (HPβCD) on fish since the use of HPβCD dramatically increased; for example, it is the active ingredient in Febreeze®. HPβCD is amphiphilic, toroidal in shape, and able to form non-covalent inclusion complexes with a variety of non-polar guest molecules. HPβCD has been shown to reduce volatility, as well as improve the aqueous solubility of guest compounds. With increasing potential for entering the environment through wastewater treatment plant (WWTP) effluent, HPβCD poses an unknown risk to non-target aquatic biota. As a result, a 145-day chronic full-lifecycle exposure, including a 30-day breeding period, using American flagfish (*Jordanella floridae*) was completed. Flow-through concentrations of 0 (control), 5, 16, 50,160, 500 and 1600 $\mu g \cdot L^{-1}$ of HPβCD were maintained via a peristaltic pump. No significant differences were observed

in growth, condition factor (K) and hepatosomatic index (HSI) when chronically exposed to HPβCD ($p \leq 0.05$). An increase in female gonadosomatic index (GSI) occurred in those exposed to HPβCD ($p \leq 0.05$). A reduction in time to reach steady-state spawning occurred at 1600 $\mu g \cdot L^{-1}$ of HPβCD, while an increase in total number of days eggs were laid was observed at 16, 160 and 1600 $\mu g \cdot L^{-1}$ of HPβCD ($p \leq 0.05$). A temporary reduction in offspring total length occurred at 21 days post-hatch at 5, 16, 50, 160 and 500 $\mu g \cdot L^{-1}$ of HPβCD ($p \leq 0.05$). Finally, larval offspring from parents exposed to HPβCD showed a moderate 3-fold decrease in tolerance to acute copper toxicity.

Effects of exposure to a model dioxin-like compound on the transcriptome of white sturgeon (*Acipenser transmontanus*) (PO)

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Exposure to dioxin-like compounds (DLCs) can cause adverse effects in fishes through activation of the aryl hydrocarbon receptor (AhR) pathway. While there is a host of information regarding the effects of DLCs to modern fishes such as salmonids or cyprinids, little is known about the specific molecular mode of action and resulting physiological impacts of DLCs to sturgeons or other ancient species of fishes. With the aim of identifying specific molecular toxicity-initiating events as a basis for hypothesizing novel adverse outcome pathways for DLCs in fishes, white sturgeon (*Acipenser transmontanus*) were exposed to the model AhR agonist, B-naphthoflavone (BNF), and transcriptional responses were evaluated by use of Illumina RNAseq after *de novo* assembly of a reference transcriptome. Abundances of greater than 2,000 transcripts were altered by at least 2-fold in livers of white sturgeon exposed to BNF as compared to controls. Abundances of transcripts of genes known to be regulated by the AhR, including those encoding proteins that catalyse Phase I, II and III metabolism of xenobiotics, were greater in livers from sturgeon exposed to BNF. In addition, abundances of transcripts of genes from pathways not known to be involved in activation of the AhR were significantly up- or down-regulated. For example, abundances of transcripts of genes involved in responses to low concentrations of oxygen, such as aryl-hydrocarbon receptor nuclear transporter (ARNT) and hypoxia-inducible factor 1 (HIF1a), were lesser in livers from sturgeon exposed to BNF. This could be indicative of potential synergistic effects of sturgeon co-exposure to DLCs and hypoxia. Overall, next-generation sequencing technologies such as Illumina could prove useful in the discovery of novel biological responses to contaminants in non-model species.

Interactive Sessions

"Ecotox 101" Session (PL)

Ecotox 101 was designed to be an educational session for ATW attendees who may not be familiar with the details of experimental work and how the collected data are used. This interactive session was held on Monday morning and was chaired by Jamey Smith (Huntsman Marine Science Centre). Two formal presentations were made, followed by an open discussion. Ken Doe (retired from Environment Canada) discussed the basics of conducting laboratory studies to determine the biological effects of contaminants on aquatic species, using pesticides as an example. Les Burridge (retired from Fisheries and Oceans Canada) discussed how these data are used in developing and supporting regulations within the Canadian federal government context. He also used pesticides as an example.

Approximately 40 people were in attendance from a wide range of backgrounds. A very good discussion took place, with many questions and comments from the floor. When asked about the value of the session, responses were uniformly positive. Several of those in attendance suggested that more time be set aside for this type of session and that a case-study format, similar to the pesticide focus of this session, be followed.

Unconventional Oil and Gas Forum (PL)

The Unconventional Oil and Gas Forum was held on the afternoon of October 8, with a format of invited speakers and an open question and answer period following the presentations. This format was selected to draw interest, provide scientific information, and foster discussion regarding this relatively new and rapidly expanding part of the energy sector in North America. Co-chaired by Marc Skinner (Stantec) and Matt Kinnie (Stantec), the forum included six speakers who provided information on the industry sector, rules and regulations in place to guide the industry and to protect the environment, and interactions and challenges related to aquatic resources.

The forum was well attended (more than 100 participants in the audience) and, based on comments from participants and interactions with the speakers, well received. The six presenters each gave a 20-minute platform talk with slides, followed by an open discussion and question period with a roving microphone, which ran for about 40 minutes.

The presenters and their topics were as follows:

The unconventional oil and gas industry: Natural gas development in New Brunswick
Sheri Sommerville, Canadian Association of Petroleum Producers

Protection of water resources: The New Brunswick "rules"
Annie Daigle, Government of New Brunswick

Potential impacts of shale gas exploration and development on water resources
Tom Al, University of New Brunswick

Fate and effects of chemicals used in shale gas extraction

Ulysses Klee, Stantec

Water and wastewater treatment for shale oil and gas sites

Paul Sanford, Stantec

Water management solutions in industry

Roy Hartstein, Southwestern Energy

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